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Effluent Monitoring Procedures
Basic Laboratory Skills
Student Reference Manual

Charles County Community Coll, La Plata, Md

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EFFLUENT-MONITORING PROCEDURES: BASIC LABORATORY SKILLS

STUDENT REFERENCE MANUAL

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OFFICE OF WATER PROGRAM OPERATIONS

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The Basic Laboratory Skills Manual is designed for the treatment plant operator or technician who is required to monitor effluent discharges under a National Pollutant Discharge Elimination System (NPDES) permit and who had little or not previous experience in laboratory work. The manual to be used by the student contains a review of basic mathematics which includes the metric system, whole numbers, decimals, formulas and percentage. The chemical laboratory sections contains applications such as weighing techniques, use of laboratory equipment, and preparation and standardization of reagents. An introduction to basic microbiological techniques is also included.

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EFFLUENT MONITORING PROCEDURES

BASIC LABORATORY SKILLS

STUDENT REFERENCE MANUAL

Awarded To:
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EFFLUENT MONITORING PROCEDURES

BASIC LABORATORY SKILLS

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PREFACE

This Course is designed for the treatment plant operator or technician who is required to monitor effluent discharges under a National Pollutant Discharge Elimination System (NPDES) Permit and who has had little or no previous experience in laboratory work.

The Course will include a review of basic mathematics. Applications in the chemical laboratory such as weighing techniques, use of equipment and solution preparation will be stressed. An introduction to basic microbiological techniques will also be included.

This manual has been prepared on a modular basis. Some of the information such as weighing techniques and solution preparation has been designed in a Standard Operating Job Procedure (SOJP) format. It is believed that this "step sequence" presentation is easier to follow in an actual laboratory procedure.

DISCLAIMER

Reference to commercial products, trade names, and manufacturers is for purposes of example and illustration. Such references do not constitute endorsement by the Charles County Community College or the U. S. Environmental Protection Agency.

EFFLUENT MONITORING PROCEDURES

BASIC LABORATORY SKILLS

STUDENT REFERENCE MANUAL

MODULE I

BASIC MATHEMATICS

In the calculation of results for the parameters of the permit system, several mathematical calculations are necessary. This module will review the following areas: Metric System, Whole Numbers, Decimals, Formulas and Percentages.

A. The Metric System

The metric system is a decimal system of units for measurements of mass, length, volume and other physical constants. It is built around a set of basic units and uses factors of 10 to express larger or smaller quantities of these units. The metric system is used virtually around the world. The United States is one of the last countries to convert to this system. It is estimated that within ten years, all of our measurements will be expressed in metric units.

Table A.1 shows the measurements that will be used in this course as well as the common terms of the English and Metric Systems.

Table A.1

Measurement	Metric	English
Weight	milligrams, grams, kilograms	ounces, pounds, tons
Length	centimeters, millimeters, kilometers	inches, feet, yards, miles
Volume	milliliters, liters	pints, quarts, gallons
Temperature	Centigrade (°C)	Fahrenheit (°F)

To express larger or smaller quantities, prefixes are added to the names of the units. For example kilo added to the word gram to give kilogram. These prefixes represent multiples of 10, making the metric system a total decimal system of measurements.

Some of the more commonly used prefixes are:

kilo-------------------one thousand (1000) times the unit expressed.
centi-------------------one-hundredth (1/100 or 0.01) of the unit expressed.
milli-------------------one-thousandth (1/1000 or 0.001) of the unit expressed.

Examples:

1 kilometer = 1000 meters
1 gram = 1000 milligrams
1 liter = 1000 milliliters

Numerical equivalents of English measurements in the Metric System are given in Table A.2. Accepted abbreviations are given in parentheses.

Table A.2

Weight	Length	Volume
1 pound =453.6 grams(g)	1 inch = 2.54 centimeters(cm)	1 quart = 946 milliliters(ml) 1 gallon= 3.78 liters (1)

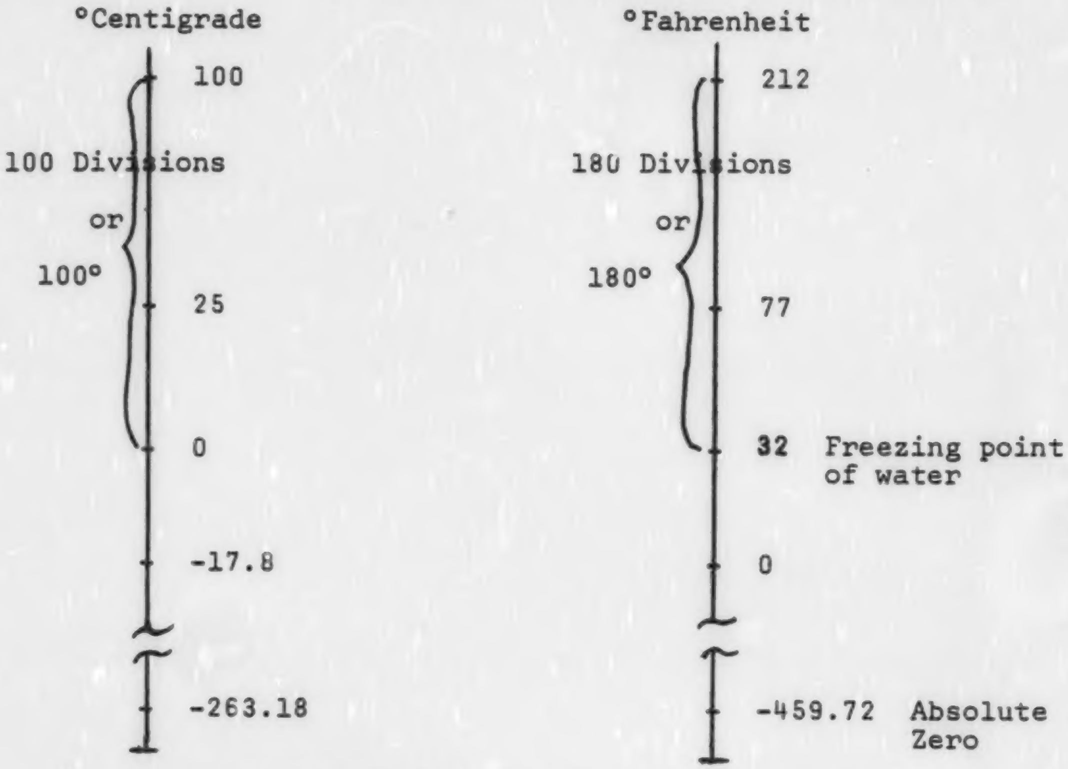
The standard unit length in the metric system is the meter. A meter is 39.37 inches, a little longer than 1 yard. One meter contains 100 centimeters or 1000 millimeters. A kilometer contains 1000 meters. The standard unit of weight is the kilogram. A kilogram contains 1000 grams. Comparing these weights to our pound (16 ounces), we find that 453.6 grams equal 1 pound and 1 kilogram is equal to 2.2 pounds. As with length measurement, prefixes are used to indicate larger and smaller units of the gram. The standard unit of volume in the metric system is the liter. The nearest common value comparable to the liter is a quart; 1.00 liter equals 1.057 quarts (or 946 ml = 1 quart). The most commonly used smaller unit of a liter is the milliliter, where 1 liter equals 1000 ml.

Two temperature scales commonly used in routine laboratory work are the Celsius (centigrade) scale, and the Fahrenheit scale. A unit of temperature on each of these scales is called a degree. The symbol for the degree is ° and it is placed as a superscript after the number and before the temperature scale indicated. Thus 100°C means 100 degrees centigrade. The centigrade scale is based on dividing the interval between the freezing and boiling temperatures of water into 100 equal parts or degrees. The freezing point of water is assigned a temperature of 0 degrees and the boiling point of water a temperature of 100 degrees centigrade. In the Fahrenheit scale, there are 180 degrees between the freezing and boiling temperatures of water. On this scale the freezing point of water is 32 degrees and the boiling point is 212 degrees Fahrenheit.

Abbreviations for these units of temperature are:

degrees Celsius (centigrade) = °C
degrees Fahrenheit = °F

The two scales are shown in Figure A.3.



Examining the temperature scales, one can see that there are 100 degrees between the freezing point and boiling point of water on the centigrade scale, and 180 degrees on the Fahrenheit scale. Then, for each 1°C increase in temperature, the °F increases 1.8°F. Conversions between the temperature scales may be done using the following conversion formulas:

$$^\circ F = 9/5\ ^\circ C + 32$$
$$^\circ C = 5/9\ (^\circ F - 32)$$

EXERCISES

1. What is the abbreviation for milliliter?

2. What is the numerical value of the following prefixes?

    kilo ______

    centi ______

    milli ______

3. What is the approximate temperature in °C if the Fahrenheit reading is 68°F?

4. Which is greater?

    1 liter or 1 quart

    1 yard or 1 meter

5. List one Metric and one English unit for mass or weight.

Lesson 2. Whole Numbers

A whole number is a digit from 0 to 9, or a combination of digits, such as 15, 324, 2, 7241. The place value in numbers are shown in Table B.1.

Table B.1

Hundred millions	Ten millions	Millions	Hundred thousands	Ten thousand	Thousands	Hundreds	Tens	Ones
6	5	2	7	1	4	2	2	3
Millions			Thousands			Ones		

The number is read: six hundred fifty-two million, seven hundred fourteen thousand, two hundred-twenty three. Notice that the word "and" is never used in reading a whole number.

To illustrate the accuracy of a certain measurement in the laboratory, it is often necessary to round off numbers. In rounding off a number to a required number of places, keep as many figures to the left as are needed. Drop the other figures and replace them with zeros. Examine the digit that is one place to the right of the last figure required and follow these rules:

(1) If this digit is less than 5, do not change the last digit retained.

Examples:

912 rounded to the nearest ten is 910
621 rounded to the nearest hundred is 600
3,498,461 rounded to the nearest million is 3,000,000

(2) If this digit is greater than five, add one to the last digit retained.

Examples.

47 rounded to the nearest ten is 50.
868 rounded to the nearest hundred is 900.
16,945 rounded to the nearest thousand is 17,000.

(3) If this digit is exactly five, the last digit retained is rounded off by increasing it one number.

Examples.

65 rounded to the nearest ten is 70
75 rounded to the nearest ten is 80.
37,500 rounded to the nearest thousand is 38,000.

Addition

In adding a series of numbers, begin with the column at the right. If the sum of a column of digits is ten or larger, carry the tens digit and add it to the sum of the digits in the next column to the left. The plus sign (+) indicates addition. Always check your work.

Example.

$$\begin{array}{rrrrl} {}^{+1} & 2 & 8 & 6 & \uparrow \\ & & 3 & 2 & \\ \hline & 3 & 1 & 8 & \end{array} \qquad \begin{array}{c} \text{Check} \\ \begin{array}{rrrrl} & 2 & 8 & 6 & \downarrow \\ +1 & & 3 & 2 & \\ \hline & 3 & 1 & 8 & \end{array} \end{array}$$

Addition can always be checked by adding down as shown in the above example.

Units such as grams, milliliters, centimeters, etc, are usually encountered in laboratory work. It is therefore necessary to note that, the units associated with a number should be carried into the final answer.

Example

$$\begin{array}{rrrl} {}^{+1}1 & {}^{+1}6 & 2 & \text{grams} \uparrow \\ & 3 & 8 & \text{grams} \\ \hline 2 & 0 & 0 & \text{grams} \end{array} \qquad \begin{array}{c} \text{Check} \\ \begin{array}{lrrrl} +1 & 1^{+1} & 6 & 2 & \text{grams} \downarrow \\ & & 3 & 8 & \text{grams} \\ \hline & 2 & 0 & 0 & \text{grams} \end{array} \end{array}$$

Also, the unit of each number must be the same in performing these operations.

Subtraction

Subtraction is the opposite of addition. When we subtract we take part of a group away from the size of the group that is left. The minus sign (-) indicates subtraction.

Addition can be used to check the subtraction operation.

Example

$$\begin{array}{r} 50 \text{ ml} \\ -12 \text{ ml} \\ \hline 38 \text{ ml} \end{array} \qquad\qquad \begin{array}{r} 50 \text{ ml} \\ -12 \text{ ml} \\ \hline 38 \text{ ml} \\ \hline 50 \text{ ml} \end{array}$$

Multiplication

Multiplication is a short method of adding a number to itself a given number of times.

Example

3 times 20 means 20 + 20 + 20 or 60

or

$$\begin{array}{r} 20 \\ \times\ 3 \\ \hline 60 \end{array}$$

A knowledge of the multiplication tables from two to twelve is necessary. The sign of multiplication is (X). Each digit must be kept in its proper column at all times. Multiplication may be checked by interchanging the numbers.

Examples

$$\begin{array}{r} 424 \\ \times\ 28 \\ \hline 3392 \\ 848 \\ \hline 11872 \end{array} \qquad\qquad \begin{array}{r} \text{Check} \\ 28 \\ \times\ 424 \\ \hline 112 \\ 56 \\ 112 \\ \hline 11872 \end{array}$$

$$\begin{array}{r} 62 \\ \times\ 10 \\ \hline 00 \\ 62 \\ \hline 620 \end{array}$$

NOTE: The product of any number multiplied by zero is zero.

In this example a short cut may be used.

Example

```
  62ml
 X 10
 620ml
```

Always be sure that numbers are kept in the proper columns and the units carried to the final answer.

Division

Division is the opposite of multiplication.

The sign of division is (÷) such as 8 ÷ 2. A fraction also indicates division: such as 8/2 or $\frac{8}{2}$.

Division should be checked by multiplication.

Examples

```
                          50grams
250 grams/5 means     5 )250grams
                         25
                          0
```

Check

```
 50 grams
X 5
250 grams
```

```
                          138
6532/47               47 )6532
                          47
                          183
                          141
                           422
                           376
                            46
```

In this example 46 is referred to as the remainder and should be expressed as a fraction.

$138\ \frac{46}{47}$

```
Check:  138
       X 47
        966
        552
       6486
     +   46
       6532
```

EXERCISES

1. Round the following numbers to the nearest hundred:
   - a. 952
   - b. 2304
   - c. 7248
   - d. 10,263
2. Round the following numbers to the nearest thousand:
   - a. 12,826
   - b. 5,556
   - c. 97,442
3. Perform the indicated operations:

a. $\begin{array}{r} 155\text{ gr.} \\ \underline{+\ 24}\text{ gr.} \end{array}$

b. $\begin{array}{r} 962\text{ gr.} \\ \underline{+\ 98}\text{ gr.} \end{array}$

c. $\begin{array}{r} 261\text{ gr.} \\ \underline{-\ 79}\text{ gr.} \end{array}$

d. $\begin{array}{r} 2478 \\ \underline{-\ 984} \end{array}$

e. $\begin{array}{r} 6{,}821 \\ \underline{-\ 1{,}987} \end{array}$

f. $\begin{array}{r} 554\text{ gr.} \\ \underline{\times\ 24} \end{array}$

g. $\begin{array}{r} 254\text{ ml} \\ \underline{\times\ 50} \end{array}$

h. $\begin{array}{r} 5{,}242 \\ \underline{\times\ 162} \end{array}$

i. $\begin{array}{r} 14{,}004 \\ \underline{\times\ 550} \end{array}$

j. 12454/65

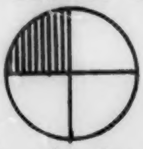
k. 1368 gr./3

l. 654 ÷ 24

m. 3481 ÷ 21

Lesson 3. Decimals

A decimal or decimal fraction is a special kind of fraction whose denominator is 10,100, 1000, 10,000 or any higher power of 10. A fraction represents a part of one whole thing. A fraction indicates that something has been cut or divided into a number of equal parts. For example, the following circle is divided into four equal parts.



One of the parts of the circle is shaded. This part may be represented by the fraction 1/4. The remaining parts may be represented by the fraction 3/4. The top part of the fraction is referred to as the numerator and the bottom part of the fraction is called the denominator.

3 ----Numerator

—

4 ----Denominator

To illustrate a fraction as a decimal, let us look at several examples:

$$\frac{2}{10} \text{ means } 2 \div 10 \text{ or } \underline{0.2}$$

$$\frac{4}{100} \text{ means } 4 \div 100 \text{ or } \underline{0.04}$$

The place value in decimals is shown in Table C.1

Table C.1

	Tenths	Hundredths	Thousandths	Ten-thousandths	Hundred-thousandths	Millionths
	.9	4	7	2	4	6

In the above number it can be seen that each digit has a specific place value. i.e. .9 means 9/10, .04 means 4/100, .007 means 7/1000 etc. All the numbers could be expressed as fractions, and added together. This would give the number shown.

Again as with whole numbers decimals must also be rounded off to indicate the accuracy of certain measurements. The same rules for whole numbers also apply to decimals.

Examples.

4.1073 rounded to the nearest tenth is 4.1.
8.47 rounded to the nearest tenth is 8.5.

Note that all decimals are dropped to the right of those required.

80.5123 rounded to the nearest thousandth is 80.512.
0.00754 rounded to the nearest thousandth is 0.008.

Addition

Decimals are added in the same way that whole numbers are added. Since only like decimals can be added, that is hundredths to hundredths, and tenths to tenths, the numbers are arranged in a vertical column with the decimal points directly below one another.

Examples.

$$\begin{array}{rl} 4.6701 & \text{grams} \\ +\ 0.7430 & \text{grams} \\ \hline 5.4131 & \text{grams} \end{array}$$

$$\begin{array}{r} 0.284 \\ 5.6 \\ +\ 25 \\ \hline \end{array}$$

In this example zeros may be added in order to keep the numbers in the proper columns.

$$\begin{array}{r} 0.284 \\ 5.600 \\ +25.000 \\ \hline 30.884 \end{array}$$

Subtraction

Decimals are subtracted in the same way that whole numbers are subtracted.

Examples.

$$\begin{array}{rl} 48.5 & \text{ml} \\ -\ 19.7 & \text{ml} \\ \hline 28.8 & \text{ml} \end{array} \qquad \begin{array}{r} 6.20 \\ -\ 0.43 \\ \hline 5.77 \end{array}$$

Answers may also be checked by addition.

Multiplication

In multiplying decimals multiply as you do whole numbers. Then, starting at the right, mark off as many places in the product as there are in the number's multiplied.

Examples.

$$\begin{array}{rl} 2.46 & \text{(2 decimal places)} \\ \text{X} \quad .8 & \text{(1 decimal place)} \\ \hline 1.968 & \text{(3 decimal places)} \end{array}$$

$$\begin{array}{rl} 26.421 & \text{(3 decimal places)} \\ \text{X} \quad 42 & \text{(0 decimal places)} \\ \hline 52\ 842 & \\ 1056\ 84\ \ & \\ \hline 1109.682 & \text{(3 decimal places)} \end{array}$$

$$25.43 \text{ grams X } 100 = \begin{array}{rl} 25.43 & \text{grams} \\ \text{X} \quad 100 & \\ \hline 00\ 00 & \\ 000\ 0\ \ & \\ 2543\quad\ \ & \\ \hline 2543.00 & \text{grams} \end{array}$$

or

$$\begin{array}{rl} 25.43 & \text{grams} \\ \text{X} \quad 100 & \\ \hline 2543.00 & \text{grams} \end{array}$$

Always be sure that the numbers are kept in the proper columns.

Division

When dividing a decimal by a decimal, the following steps should be followed.

1) Move the decimal point of the divisor to the right of the last digit and indicate where the new decimal point will be.

2) Move the decimal point of the dividend to the right the same number of places as the decimal point was moved in the divisor, annexing zeros if necessary.

3) Place the decimal point in the quotient directly above the new decimal point in the dividend.

4) Divide in the same way as in the division of whole numbers.

Examples.

```
                          4.14←—Quotient
36.85/8.9  =      8.9 )36.850 ←—Dividend
             Divisor   35 6
                        1 25
                          89
                         360
                         356
                           4
```

Check

```
   4.14
X   8.9
   3726
  3312
  36.846
+   .004
  36.850
```

The number of places that the answer should be carried out is governed by the accuracy of the measuring devices.

```
                          .147 grams
42 grams/284  =   284 )42.000
                       28 4
                       13 60
                       11 36
                        2 240
                        1 988
                          252
```

0.147 rounded to the nearest hundredth would be 0.15 grams.

EXERCISES

1. Round the following numbers to the nearest tenth
    - a. 26.423
    - b. 2.15
    - c. 624.91
2. Round the following numbers to the nearest thousandth:
    - a. 0.6894
    - b. 0.00219
    - c. 64.23555
3. Perform the indicated operations:

a. $\begin{array}{r} 5.62 \text{ gr.} \\ \underline{+\ .29} \text{ gr.} \end{array}$ b. $\begin{array}{r} 124.89 \text{ gr.} \\ \underline{+\ \ 9.78} \text{ gr.} \end{array}$ c. $\begin{array}{r} 48.7 \text{ ml} \\ \underline{-\ 9.8} \text{ ml} \end{array}$

d. $\begin{array}{r} 24.6291 \text{ gr.} \\ \underline{-21.9764} \text{ gr.} \end{array}$ e. $\begin{array}{r} 24.62 \\ \underline{\times\ \ \ \ 21} \end{array}$ f. $\begin{array}{r} 264 \text{ ml} \\ \underline{\times\ 10} \end{array}$

g. $\begin{array}{r} 0.624 \\ \underline{\times\ \ \ .48} \end{array}$ h. 2.431/0.8 i. 92/104

j. 144.2/.04

Lesson 4. Formulas

A formula is an equation that is used in solving mathematical expressions. In using formulas, combinations of whole numbers and decimals are included. The operations of addition, subtraction, multiplication and division are also included. An equation is a statement that one expression is equal to a second expression.

Examples.

$a + b = c$ is an equation

If $a = 4$ and $b = 3$ then $c = 7$. Expressed as an equation then $4 + 3 = 7$. One side of an equation must always equal the other side. Remember also that the equation tells which mathematical operations must be performed.

Mixed Operations

To solve examples containing parentheses, do the work within the parentheses and proceed in the usual way. Within parentheses and in examples without parentheses do multiplication from left to right before doing additions and subtraction.

Examples.

1. $3 \times 6 - 2$

$$18 - 2 = 16$$

If the subtraction had been performed first then the answer would have been 12, which is incorrect.

2. $54 + [20 - (7 + 3)] =$

$$\begin{aligned} &= 54 + [20 - (10)] \\ &= 54 + [20 - 10] \\ &= 54 + 10 \\ &= \underline{5.4} \end{aligned}$$

3. $\dfrac{(24 - 3) \times 6}{2}$

   a) $\dfrac{21 \times 6}{2}$

Next multiplication or division may be performed

b) Multiplication 1st $\frac{126}{2} = 63$

c) Division 1st $\frac{21 \times \overset{3}{\cancel{6}}}{\underset{1}{\cancel{2}}} = 63$

Cancelling factors in step 3 is an allowable short cut. It should also be mentioned that cancellation of units may be done simultaneously.

4. $\frac{(221 - 62)\ \ (24)}{6}$

$$= \frac{(159)\ (\overset{4}{\cancel{24}})}{\underset{1}{\cancel{6}}} = 636$$

5. Given the following equation

$$A = \frac{(B-C)}{D}$$

and the values

$B = 24{,}736$mg
$C = 24{,}720$mg
$D = 0.05$ liter

Solve the equation for A.

a) $A = \frac{(24{,}736 \text{ mg} - 24{,}720 \text{ mg})}{.05 \text{ liter}}$

$$= \frac{16 \text{ mg}}{.05 \text{ liter}}$$

$$= 320 \text{ mg/liter}$$

Note how the units were carried all the way to the final answer.

EXERCISES

1. Solve the following expressions:

   a. $21 + (62 - 3) =$

   b. $(6 - 2)\,(12 - 9) =$

   c. $(21 \times 3) \div (6 + 2) =$

   d. $\frac{(22.4 - 21.1)\,(100)}{50} =$

2. Given the following formula:

   $A = \frac{(B-C)\,(1000)}{D}$

   Find A when B = 24.6239 grams
   C = 24.6197 grams
   D = 50 ml

3. Given the formula:

   $A = B \times C/D$

   Find A when B = 20.1 ml
   C = 0.0375 N
   D = 15.0 ml

Lesson 5. Percentage

Percentage is a term used to denote that a whole quantity divided into 100 equal parts is taken as the standard of measure. The word percent means per hundred. The percent sign (%) is the symbol for percentage.

Examples.

1. A ballplayer had 30 hits per 100 hundred times at bat. Therefore he had 30/100 (30 per hundred) or

$$\frac{30}{100} \text{ X } 100 = 30\%$$

Note!! The expression was multiplied by 100 to arrive at a % value.

2. A hunter hit 150 bullseyes out of 300 shots. Therefore

$$\frac{\overset{50}{\cancel{1}\cancel{5}\cancel{0}}}{\underset{\underset{1}{\cancel{2}}}{\cancel{3}\cancel{0}\cancel{0}}} \text{ X } \cancel{1}0\cancel{0} = 50\%$$

Efficiency measurements are usually expressed in terms of percentage such as:

220 pounds of pollutants were coming into a treatment plant and 20 pounds were being discharged. What is the efficiency of the plant?

$$\text{Then: } \frac{220 - 20}{220} \text{ X } 100 = \text{\% Efficiency}$$

$$= \frac{200}{220} \text{ X } 100$$

$$= .91 \text{ X } 100 = 91\%$$

% Efficiency is sometimes referred to as % removal.

A basic equation for calculation of % removal is:

$$\% \text{ Removal} = \frac{(A-B)}{A} \times 100$$

A = Amount or concentration of a particular constituent in the influent.

B = Amount or concentration of a particular constituent in the effluent.

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EXERCISES

1. Express the following decimals as percentages.

   a. 0.02

   b. 0.92

   c. 0.41

2. Solve the following expressions

   a. $\frac{450 - 21}{450} \times 100$

   b. $\frac{121 - 10}{121} \times 100$

   Express the answers as percentages.

ANSWERS

Module 1, Lesson 2

The Metric System

1. ml.

2. kilo = 1,000
   centi = 1/100 or 0.01
   milli = 1/1000 or 0.001

3. 20°C

4. 1 liter
   1 meter

Lesson 2

Whole Numbers

1. a. 1,000
   b. 2,300
   c. 7,200
   d. 10,300

2. a. 13,000
   b. 6,000
   c. 97,000

3. a. 179 gr.
   b. 1,060 gr.
   c. 182 gr.
   d. 1,494
   e. 4,834
   f. 13,296 gr.
   g. 12,700 ml.
   h. 849,204
   i. 7,702,200
   j. 191 35/65
   k. 456 gr.
   l. 27 1/4
   m. 165 16/21

ANSWERS

Module 1, Lesson 3

Decimals

1. a. 26.4
   b. 2.2
   c. 624.9

2. a. 0.689
   b. 0.002
   c. 64.236

3. a. 5.91 gr.
   b. 134.67 gr.
   c. 38.9 ml.
   d. 2.6527 gr.
   e. 517.02
   f. 2640 ml.
   g. .2995
   h. 3.038
   i. .884
   j. 36.05

Lesson 4

Formulas

1. a. 80
   b. 12
   c. 7.87
   d. 2.6

2. 0.084 grams/ml

3. 0.05 N

Lesson 5

Percentage

1. a. 2%
   b. 92%
   c. 41%

2. a. 95%
   b. 91%

MODULE II

CHEMICAL LABORATORY

The analyses required under the permit system, will for the most part be performed in a chemical laboratory. The sections that follow will cover the basics of the chemical laboratory.

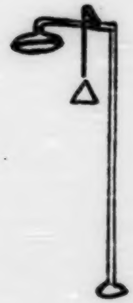
A. Safety
B. Bench Sheets and Notebooks
C. Labeling
D. Names and Formulas of Compounds
E. Care and Use of Equipment
F. Matter
G. Solutions
H. Use of Laboratory Balances
I. Volumetric Analysis

The format used for part of this section is referred to as Standard Operating Job Procedure (SOJP). It has been modified somewhat to meet the needs of this laboratory course.

Lesson 1. Laboratory Safety

Learning laboratory safety habits is something like learning to drive an automobile safely - at the start it takes deliberate effort, but when properly learned it becomes almost second nature. Safe practice in the laboratory requires hardly any more effort than unsafe practice, and the important results are prevention of injury or damage. The laboratory is provided with equipment designed to help prevent accidents from occuring and to prevent or reduce injury or damage if accidents should occur. You must use the equipment provided for the prevention of accidents (such as safety glasses and fume hoods, etc.). It is also necessary to know the location and understand the operation of safety equipment for damage reduction due to accidents (such as fire extinguishers, eye washes, etc).

The safety equipment and procedures for operating follow in the next section.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. SAFETY			
1. Safety Shower Burning Clothing or Clothing Wet with Corrosive Liquid 	1. Pull on the chain or ring (as shown in the diagram).	1a. Water will flow at a very high rate and continue to flow after you release the ring. 1b. If eyes are affected, look up toward the shower head to flush them. 1c. If clothing is soaked with toxic or corrosive material, remove your clothes.	I.
2. Eye Wash Corrosive or Irritating Chemical in Eyes. 	1. Remove safety glasses and bend over until your eyes are directly in the dual streams of water.	1a. Hold eyes open with your hands.	II.
	2. Rinse thoroughly until all of foreign material is removed.	2a. 15 minutes is the recommended minimum time.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Fire Extinguishers Carbon Dioxide and Dry Chemical Type.	1. Remove the unit from its mount and bring it close to the fire area.	1a. Carbon dioxide extinguishers may be used on both electrical and chemical fire. 1b. If a pool of burning liquid is involved, the dry chemical type should be used. 1c. Fire hoses and "soda-acid" extinguishers are good for paper, wood or other common combustibles but are not good choices for some chemical fires and would never be used on electrical fires.	III.
	2. Pull the retainer ring. 3. Point horn at the base of the fire. 4. Squeeze the handle.		
4. Fire Blanket Burning Clothing	1. Pull rope on side of cabinet. 2. Begin unwinding blanket and wrapping person securely inside.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
5. General First Aid Kit - Minor Burns and Cuts.	1. General first aid instructions will be available on kit and should be followed.	1a. In addition to treatment of minor burns and cuts, the kit should contain medication to neutralize the effects of some toxic materials. It should also contain chemicals to induce vomiting as well as substances to help revive fainting victims.	
6. Fume Hood Venting of Toxic, Flammable and Unpleasant Vapors and Dust.	1. Check air exhaust system.	1a. The hood as shown is similar to most laboratory benches. It will include a sink, water spigots, gas and electrical services. The hood area is enclosed on three sides by a solid barrier and at the front by a safety glass shield which may be raised or lowered.	IV.
	2. Turn on fan and close front shield to within one inch of bench top. 3. The air flow into the hood should now be strong enough to cause paper to "flap in the breeze" when it is held in the opening.		
7. Safety Glasses __MUST BE WORN AT ALL TIMES IN THE LABORATORY.__ 	1. Upon entering the laboratory, obtain a pair of safety glasses and __wear them__.	1a. Safety glasses are usually made of transparent plastic or of hardened safety glass. 1b. Safety goggles are made to fit over regular glasses.	

(Continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Safety Glasses (Continued) 			
8. Vapor Detection	1. Do not hold face directly over a container when noting an odor.		
	2. Fan a little of the vapor toward your nostrils by sweeping your hand over the top of the container.		
9. Acid Burns	1. Wash immediately with large amounts of cold water.		
	2. Flush with a sodium bicarbonate (baking soda) solution to neutralize the acid.	2a. A 10-20% solution is recommended. This solution can be purchased commercially.	
10. Alkali Burns	1. Wash immediately with large amounts of cold water.		
	2. Flush with a boric acid solution.	2a. A saturated solution is recommended. This solution can also be purchased commercially.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
11. Acid Dilutions	1. When diluting any concentrated acid especially sulfuric acid, pour the acid slowly into the water. NEVER reverse the order. (Steam may form with explosive violence.)		
12. Specific Regulations that Should be Followed in the Laboratory.		1. Read the label twice before taking anything from the bottle. 2. Never return unused chemicals to the stock bottle. 3. Do not insert your own pipets or medicine droppers into the reagent bottles. 4. Do not lay the stopper of the bottle down in such a manner that it picks up impurities. 5. Do not heat heavy glassware such as volumetric flasks, graduated cylinders, or bottles; they break easily.	

TRAINING GUIDE NOTES

I. Safety Showers

The valve handle of the safety shower should be rigidly fixed and plainly labeled. The valve should readily open in either direction and remain open until intentionally closed. Water flow pressure must be sufficient to drench the subject rapidly. The shower area must be kept clear of obstructions and drainage provided where possible; tempered (not cold) water of drinking purity should be used in safety showers.

II. Eye Washes

If emergency eye-wash fountains are used, they should deliver a gentle flow of clean, aerated water. A hand-held eye wash spray with a 5-foot hose is more adaptable to unusual situations, including head and body splashes, but should not be located where it can be contaminated by waste materials. Eye wash devices or hoses should be flushed at least weekly. It must be understood by all that eye protection is infinitely more important than eye washes.

III. Fire Extinguishers

Fire extinguishers in the laboratory should be appropriate (up to 10-lb. charge) for rapid use. There should be at least one for each work bench. Dry Chemical (pressurized bicarbonate powder) fire extinguishers may be preferred for certain areas, but carbon dioxide is satisfactory for most small fires and is cleaner to use. Extinguishers should be recharged promptly after use or whenever regular monthly weight check indicates more than 20 percent loss of carbon dioxide. Dry chemical powder extinguishers can be repressurized when slightly below normal pressure. If, however, the pressure is substantially lower than the operating pressure, the extinguisher should be serviced to correct a leak. At least one large back-up fire extinguisher of dry chemical powder type should be conveniently located outside each laboratory.

IV. Fume Hood

Fume hoods should be sufficiently effective so that substances with strong odors, such as mercaptans and pyridine, are not detectable by the laboratory worker. A 100-linear ft/min flow at a standard height above the hood surface of the open hood is the minimum that is considered satisfactory. Flow varies markedly near the surface. (Velometers should be used to survey hoods on a regular schedule and the results posted on the hood face.) [Fans should be located on the roof so that all ductwork in the building is under negative pressure.]

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Lesson 2. Bench Sheets and Notebooks

In any laboratory analysis it is necessary that the records of all work performed be kept in a logicial order. Bench sheets are used for day to day records of specific parameters such as Biochemical Oxygen Demand (BOD), Suspended Solids, etc. A sample BOD bench sheet is shown below.

BENCH SHEET B.O.D. DETERMINATIONS

SAMPLE SOURCE &	DATE		BOTTLE	INITIAL	FINAL	DEPLETION	%	B.O.D.
DATE COLLECTED	IN	OUT	NO.	D.O.	D.O.	MG/L	DIL.	MG/L

The information that is contained on a bench sheet should include the following:

1. Date

Always record the date the sample was taken. It is further recommended that the time also be noted, since comparison with other data will be more relevant.

2. Sample Designation

This should be included so that the exact sampling point is known. It is recommended that a rough schematic of the plant be available so that the sampling points can be coded.

i.e.		
	Influent	#1
	Primary Effluent	#2
	Secondary Effluent	#3
	Final Effluent	#4

On the previous Bench Sheet, enter the following information in the proper format. A sample of final effluent was taken from the La Plata Treatment Plant at 9:00 a.m., December 19, 1974 for BOD Analysis.

The type of sample, whether it be grab or composite should also be recorded. It should also be noted whether or not a preservative was added. If it was, then the type and amount should also be written on the label.

3. Equipment Designation

The equipment that is used for a particular analysis must be coded in such a way that it reflects the exact sample. For example in the above BOD analysis, eight bottles will be used. Their numbers will indicate their identification and should correlate with the Final Effluent sample. Enter the following numbers on that sheet, 62, 114, 78, 284, 91, 22, 48, and 101. If we had been sampling all four points in the plant and used eight bottles for each point then thirty-two numbers would be recorded. Therefore it is absolutely necessary that the equipment, whether it be BOD bottles or crucibles be recorded accurately.

4. Raw Data

This type of information includes weights, volume of sample. etc. In the above example the raw data would be Initial D.O. and final D.O.

In the above sheet the following results were obtained:

	Initial D.O.	Final D.O.
#64	8.3	--
#114	--	4.2

Enter these numbers on your bench sheet.

When the results have been tabulated the bench sheet should be placed in a permanent binder for future reference.

The laboratory notebook is also a necessity for the laboratory worker. Its purpose is to have a permanent record of all phases of the analysis as it was performed. It may appear that some information is being duplicated but for the most part it will include the additional information necessary to make the analysis complete. In addition to the five points previously covered, the following information should be included in the notebook.

6. Observation of Abnormal Conditions

In a suspended solids determination accurate weights of crucibles and filter paper are absolutely necessary. If the type of balance used in the analysis is changed, it should be recorded in the notebook. If in the final weighing, the crucible is inadvertently touched with the hands, this should also be recorded. Many variances in final results can be found by simply referring back to this section in the notebook.

7. Calculations

In the computation of the final results in the previous analyses, the manner in which they were arrived at would be included in the notebook.

For Example.

$$\text{Depletion (mg/l)} = \text{Initial D.O.} - \text{Final D.O.}$$
$$= 8.3 - 4.2$$
$$= 4.1 \text{ mg/l}$$

$$\text{BOD (mg/l)} = \frac{\text{Depletion}}{\text{\% Dilution (as a decimal)}}$$

$$= \frac{4.1}{.50}$$

$$= 8.2 \text{ mg/l}$$

The use of bench sheet and notebooks then is designed, so that we will have a clear and concise record of everything that is pertinent to a certain analysis. Do not rely on your memory for specific details such as: Which balance did I use?

What crucible was used for influent? Where is the paper towel that had the Initial D.O. readings? The bench sheet and notebook should be the only two items that you need for your day to day record keeping.

Typical Laboratory Data Sheet
for
TOTAL SUSPENDED (NON-FILTERABLE) SOLIDS, mg/liter

Name of Plant ______________________________

STEP	SUSPENDED SOLIDS	SAMPLE	SAMPLE	SAMPLE	
B.2	Identification			INS #1	1
B.2	Type (grab, etc.)			GRAB	2
B.2	Date & Time Collected			5/1/74 0900	3
B.2	Sample Collector			Tom Sampler	4
C.4	Filter Identification			WG2	5
E.1	Date & Time Analysis began			5/1/74 1100	6
E.8	ml Sample Filtered			67.0	7
H.6	1st weight of Filter* plus Residue (g)			0.1426	8
I.10	2nd weight of Filter* plus Residue (g)			0.1416	9
I.13	Difference (1st-2nd)			0.0010	10
I.14	3rd weight of Filter* plus Residue (g)			0.1413	11
I.14	Difference (2nd-3rd)			0.0003	12
I.14	Final weight of Filter* plus Residue (g)			0.1413	13
C.7	Weight of Filter* (g)			0.1293	14
K.3	Find Difference (g) by subtracting Line 14 from Line 13			0.0120	15
K.5	Divide to 7 decimal places: $\frac{\text{(line 15) difference (g)}}{\text{(line 7) ml sample filtered}}$			0.0001791	16
K.7	Multiply Line 16 by 1000 000 (move decimal point 6 places Rt.)			179.1	17
K.9	Round answer on Line 17 to nearest whole number			179 mg/l	18
I.15	Analyst			Mary Analyst	19

*"Filter" means the filter disc if a funnel type filtration assembly is used. If Gooch crucibles are used "filter" means the crucible containing a filter disc.

Lesson 3. Labeling

When a chemical or a piece of equipment is used for a specific analysis, it should have some type of identification. When you prepare a chemical from a stock container (purchased from a supply house), you must identify that chemical properly. The stock container will have all the necessary information on its label. A general format for labeling reagent bottles is as follows.

Chemical Name
Chemical Formula
Concentration
Date **Initials**

In preparing a chemical reagent a specific procedure would be as follows:

> Prepare a sulfuric acid solution 10% by volume by pouring 10 ml of concentrated sulfuric acid (H_2SO_4), into 90 ml of distilled water. Cool the solution to room temperature and transfer to a storage bottle.

The label should be:

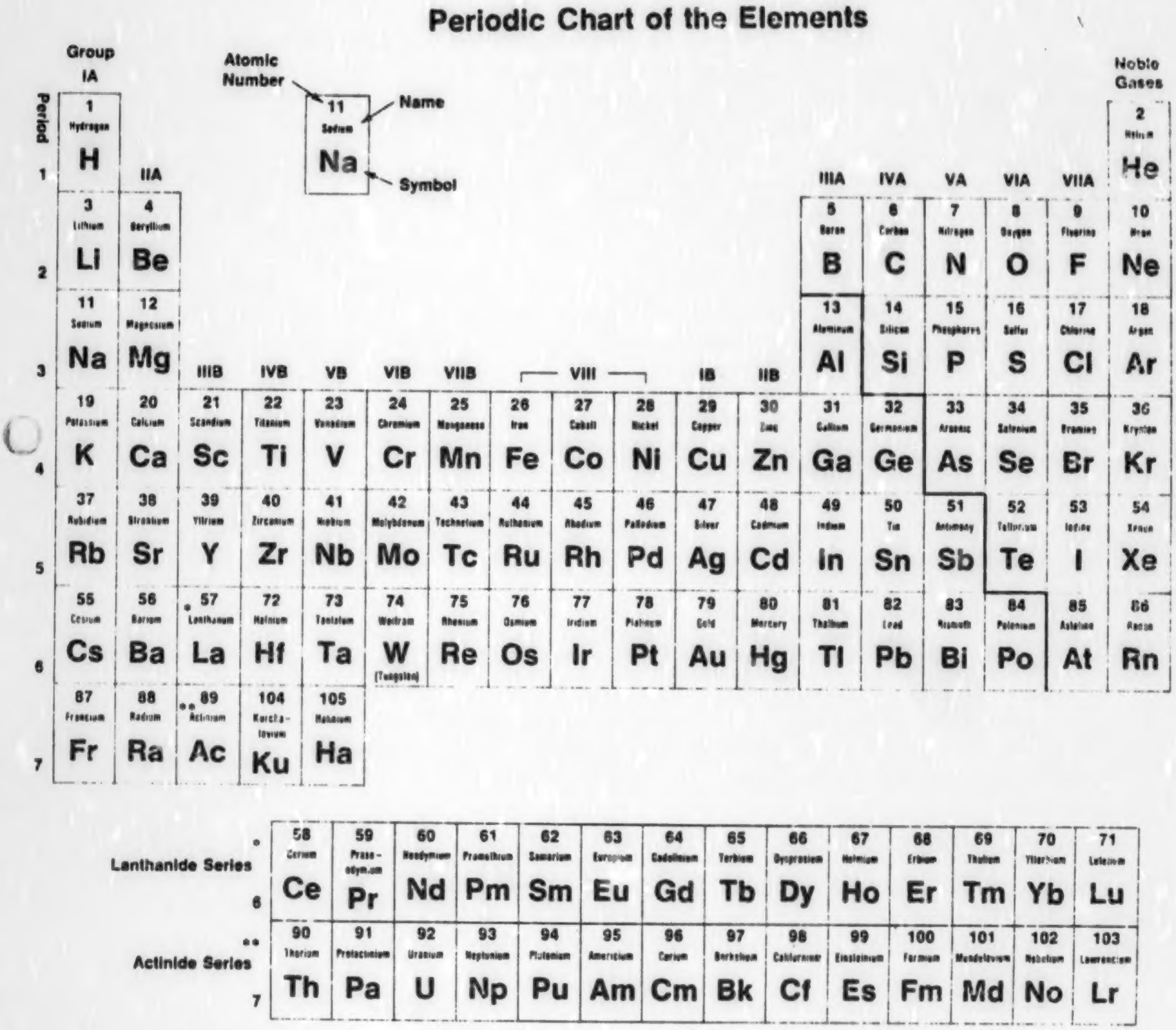
Sulfuric Acid
H_2SO_4
10% by Volume
12/19/74 WTE

All necessary information has been included on the label to properly identify it. It takes a little more time but it is well worth it in the long run.

Several labeling tools are available, and each has its place in the laboratory. Most beakers and flasks will have a hexagon space of ground glass which can be used to identify it. A lead pencil should be used for this type of marking. Grease pencils are primarily used for test tubes. It should be noted that the grease pencil marking will readily rub off. When porcelain is labeled, a special technique should be used, since the item will be repeatedly heated and cooled. An etching device such as a Vibra-Groover®, should first be used to put either a number or letter on the item. Next the etching should be filled in by rubbing it with a stick dipped in 1% Ferric Chloride ($FeCl_3$) solution (can either be prepared or commercially purchased). The porcelain crucible or other item is then placed in a muffle furnace (Approximately 600°C) and fired for 10 minutes. After cooling the porcelain is ready for use. Whatever labeling techniques you use, be consistent, and remember that the label is intended not only for convenience but also for safety.

Lesson 4. Names and Formulas of Compounds

In virtually every chemical analysis, the name and formulas of chemical compounds appear. Compounds are pure substances that are composed of two or more elements. Elements may be referred to as the basic building blocks of all substances. At present there are 105 elements known. These elements are shown in the periodic table below.*



Periodic Chart of the Elements

Key: Atomic Number 11, Name Sodium, Symbol Na

Period	Group IA	IIA	IIIB	IVB	VB	VIB	VIIB	VIII	VIII	VIII	IB	IIB	IIIA	IVA	VA	VIA	VIIA	Noble Gases
1	1 Hydrogen H																	2 Helium He
2	3 Lithium Li	4 Beryllium Be											5 Boron B	6 Carbon C	7 Nitrogen N	8 Oxygen O	9 Fluorine F	10 Neon Ne
3	11 Sodium Na	12 Magnesium Mg											13 Aluminum Al	14 Silicon Si	15 Phosphorus P	16 Sulfur S	17 Chlorine Cl	18 Argon Ar
4	19 Potassium K	20 Calcium Ca	21 Scandium Sc	22 Titanium Ti	23 Vanadium V	24 Chromium Cr	25 Manganese Mn	26 Iron Fe	27 Cobalt Co	28 Nickel Ni	29 Copper Cu	30 Zinc Zn	31 Gallium Ga	32 Germanium Ge	33 Arsenic As	34 Selenium Se	35 Bromine Br	36 Krypton Kr
5	37 Rubidium Rb	38 Strontium Sr	39 Yttrium Y	40 Zirconium Zr	41 Niobium Nb	42 Molybdenum Mo	43 Technetium Tc	44 Ruthenium Ru	45 Rhodium Rh	46 Palladium Pd	47 Silver Ag	48 Cadmium Cd	49 Indium In	50 Tin Sn	51 Antimony Sb	52 Tellurium Te	53 Iodine I	54 Xenon Xe
6	55 Cesium Cs	56 Barium Ba	*57 Lanthanum La	72 Hafnium Hf	73 Tantalum Ta	74 Wolfram W (Tungsten)	75 Rhenium Re	76 Osmium Os	77 Iridium Ir	78 Platinum Pt	79 Gold Au	80 Mercury Hg	81 Thallium Tl	82 Lead Pb	83 Bismuth Bi	84 Polonium Po	85 Astatine At	86 Radon Rn
7	87 Francium Fr	88 Radium Ra	**89 Actinium Ac	104 Kurchatovium Ku	105 Hahnium Ha													

	Period														
* Lanthanide Series	6	58 Cerium Ce	59 Praseodymium Pr	60 Neodymium Nd	61 Promethium Pm	62 Samarium Sm	63 Europium Eu	64 Gadolinium Gd	65 Terbium Tb	66 Dysprosium Dy	67 Holmium Ho	68 Erbium Er	69 Thulium Tm	70 Ytterbium Yb	71 Lutetium Lu
** Actinide Series	7	90 Thorium Th	91 Protactinium Pa	92 Uranium U	93 Neptunium Np	94 Plutonium Pu	95 Americium Am	96 Curium Cm	97 Berkelium Bk	98 Californium Cf	99 Einsteinium Es	100 Fermium Fm	101 Mendelevium Md	102 Nobelium No	103 Lawrencium Lr

* Taken from: Foundations of College Chemistry, Third Ed., by Morris Hein, Dickenson Publishing Company, Inc., Encino, California, 1973

Each element has a particular symbol. The symbol is an abbreviation for that element. The elements numbered (located above the symbol) 1 through 92 occur naturally (i.e. can be found in earth's crust, water or the atmosphere). Elements numbering 93-105 do not occur naturally but have been synthesized in small quantities in the laboratory. The symbols that are used to represent the elements are also used to represent compounds. For example the compound NaCl represents the combination of sodium (Na) (#11) and chlorine (Cl #17) and its name is sodium chloride.

All the chemical procedures that are included in this course will always refer to a compound with its formula and name together. For example: Prepare a 10% by volume sulfuric acid (H_2SO_4) solution by . . . . Weigh out 186.15 grams of sodium thiosulfate ($Na_2S_2O_3$) . . . . In several of the chemical formulas, you will note that subscripts are used. The subscript tells how many atoms of that element are contained in the compound. In water (H_2O) there are two atoms of hydrogen and one atom of oxygen. The subscripts help to differentiate one compound from another. The compound hydrogen peroxide (H_2O_2) although similar to water is obviously not the same since there are 2 atoms of oxygen in the peroxide and only 1 atom in the water.

In choosing the proper chemical for an analysis, it cannot be overemphasized that the name and formula that occur on the label of the chemical must match the name and formula in the procedure that has been given. Several names may appear to be correct because of similarities in spelling such as:

sodium sulfate Na_2SO_4 and
sodium sulfite Na_2SO_3

These are not the same. The sulfate compound has one more oxygen atom than the sulfite. Another minor spelling variation would be potassium nitrate KNO_3 and potassium nitrite KNO_2. What is the difference here?

Another variation and, in fact, a very important property of compounds, is the addition of the word anhydrous to the name. This means without water. The chemical has been prepared (at the factory) without water. If the chemical does have water in it, it will be referred to as a hydrate.

Examples.

Sodium Thiosulfate Pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$)

This means that the compound has 5 water molecules associated with it. Note that the prefixes to the word hydrate are mono, di, tri, tetra, penta, hexa, hepta, octa, nona, and deca referring to the numbers 1 through 10 respectively.

Calcium Chloride, Anhydrous ($CaCl_2$)

This means that the compound contains no water.

When choosing a chemical for a particular analysis, the stock chemical bottle must be studied very carefully. It contains a label that gives the name of the compound as well as the formula. It also contains (CAUTIONS) such as explosive, toxic (poisonous). The hazards presented by these chemicals are not evident from appearance, smell, or everyday knowledge. Hazards must be foreseen and avoided. It is safest to assume that all chemicals, even water if not safely handled, can be hazardous. Read the label completely and follow the warnings that are indicated. The label will also mention any additional storage requirements that might be necessary for a particular reagent such as [Store at 25°C]. The purity of the chemical is also indicated. Analytical or Reagent Grade is the highest purity. The amounts of impurities are shown on the label. The word ACS (American Chemical Society)also might be shown. This also means reagent grade. A lower grade of chemical would be laboratory or practical grade. Usually, amounts of impurities would not be listed on this label. A sample label is shown below.

$Na_2S_2O_3 \cdot 5H_2O$ 5 lbs.
SODIUM THIOSULFATE
(cystals)
Reagent, A. C. S.

CAUTION !!!
Emits Toxic Fumes When Heated
Keep container tightly closed.
Do not take internally.

The exercises on the following pages consist of various check lists and consumable supply lists. For every check list there is a consumable supply list. Complete these as the directions state.

CONSUMABLE SUPPLIES I

1. 480 g. manganous sulfate tetrahydrate, $MnSO_4 \cdot 4H_2O$
2. 500 g. sodium hydroxide, $NaOH$
3. 125 g. sodium iodide, NaI
4. 10 g. sodium azide, NaN_3
5. 4 plastic weighing boats
6. 1 small size spatula
7. 1 medium size spatula
8. 10 g. soluble starch
9. 10 ml chloroform
10. 186.15 g. sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$
11. 6 g. potassium biiodate (or potassium biniodate) $KH(IO_3)_2$
12. 3 g. potassium iodide, KI
13. 10 ml concentrated sulfuric acid, H_2SO_4
14. pen or pencil
15. paper (to record data)

CHECK LIST - I

Chemical Names:

Place number from "consumable" list I by matching name.

______ a. Sodium Nitrate

______ b. Sodium Thiosulfate, Anhydrous

______ c. Sodium Thiosulfate Pentahydrate

______ d. Carbon Tetrachloride

______ e. Manganese Hydroxide

______ f. Manganous Sulfate Tetrahydrate

______ g. Magnesium Sulfate Heptahydrate

______ h. Potassium Bichromate

______ i. Sodium Iodide

______ j. Sodium Fluoride

______ k. Potassium Biiodate

______ l. Sodium Sulfite

______ m. Sodium Thiosulfite

______ n. Dilute Sulfuric Acid

______ o. Sodium Azide

______ p. Sodium Acetate

______ q. Concentrated Sulfuric Acid

______ r. Soluble Starch

CONSUMABLE SUPPLIES - II

1. Small wad of cotton
2. 10 g. potassium dihydrogen phosphate, KH_2PO_4
3. 25 g. dipotassium hydrogen phosphate, K_2HPO_4
4. 35 g. disodium hydrogen phosphate heptahydrate, $Na_2HPO_4 \cdot 7H_2O$
5. 3 g. ammonium chloride, NH_4Cl
6. 25 g. magnesium sulfate heptahydrate, $MgSO_4 \cdot 7H_2O$
7. 30 g. anhydrous calcium chloride $CaCl_2$
8. 1 g. ferric chloride, $FeCl_3$
9. Manganous sulfate solution*, alkaline iodide azide solution*, starch solution*, standard sodium thiosulfate solution*, and concentrated sulfuric acid*.
10. pen or pencil
11. paper (for recording data)
12. grease pencil

* As listed in the EMP on the Winkler Determination of Dissolved Oxygen Azide modification.

CHECK LIST - II

Chemical Names:

Place number from "consumable" list by matching name.

_______ a. Calcium Chloride Dihydrate

_______ b. Sodium Chloride

_______ c. Ammonium Chloride

_______ d. Ferrous Chloride

_______ e. Potassium Dihydrogen Phosphate

_______ f. Magnesium Sulfate Heptahydrate

_______ g. Ammonium Chlorate

_______ h. Calcium Chloride, Anhydrous

_______ i. Ferric Chloride

_______ j. Dipotassium Hydrogen Phosphate

1. 721.8 mg anhydrous potassium nitrate, KNO_3

2. 5.0 g sodium arsenite, $NaAsO_2$
3. 1 g brucine sulfate, $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$
4. 0.1 g sulfanilic acid, $NH_2C_6H_4SO_3H \cdot H_2O$
5. 3 ml concentrated hydrochloric acid, HCl
6. 500 ml concentrated sulfuric acid, H_2SO_4

7. 300 g sodium chloride, $NaCl$

CHECK LIST - III

Chemical Formulae:

Place the number from the "consumable" list by the matching formula.

________ a. KNO_2

________ b. KCl

________ c. HCl

________ d. KNO_3

________ e. $NaClO_3$

________ f. $(C_{24}H_{28}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$

________ g. $NaAsO_2$

________ h. $(C_{23}H_{26}N_2O_4)_2 \cdot H_2SO_4 \cdot 7H_2O$

________ i. H_3PO_4

________ j. $NH_2C_6H_4SO_3H \cdot H_2O$

________ k. H_2SO_4

________ l. NaClO

________ m. NaCl

CONSUMABLE SUPPLIES - IV

1. 134 g potassium sulfate, K_2SO_4
2. 200 ml concentrated sulfuric acid, H_2SO_4
3. 2 g red mercuric oxide, HgO
4. 25 ml 6N Sulfuric acid, H_2SO_4
5. 500 g sodium hydroxide, NaOH
6. 25 g sodium thiosulfate pentahydrate, $Na_2S_2O_3.5H_2O$
7. 200 mg methyl red indicator
8. 100 mg methylene blue indicator
9. 150 ml 95% ethyl alcohol, C_2H_5OH
10. 20 g boric acid, H_3BO_3

CHECK LIST - IV

Chemical Formulae:

Place the number from "consumable" list by the matching formula.

_______________ a. $HBiO_3$

_______________ b. $H_2B_4O_7$

_______________ c. $NaOH$

_______________ d. $Na_2S_2O_3$

_______________ e. H_3BO_3

_______________ f. CH_3OH

_______________ g. $Na_2S_2O_3 \cdot 5H_2O$

_______________ h. Hg_2O

_______________ i. Hg_2SO_4

_______________ j. $KHSO_4$

_______________ k. HgO

_______________ l. C_2H_5OH

_______________ m. $Na_2S_2O_3 \cdot H_2O$

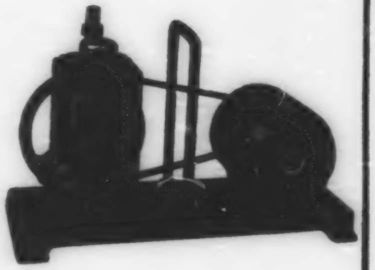
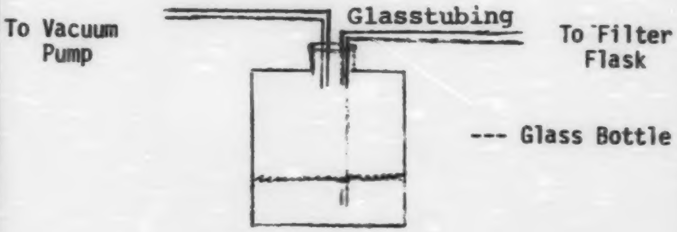
_______________ n. K_2SO_4

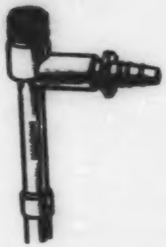
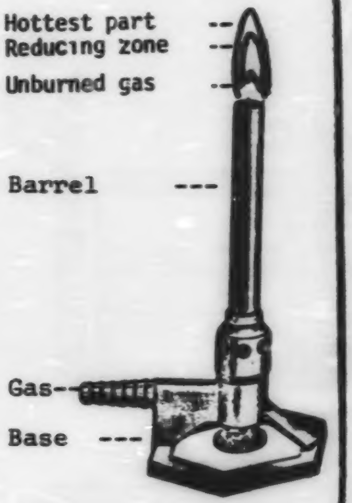
_______________ o. H_2SO_4

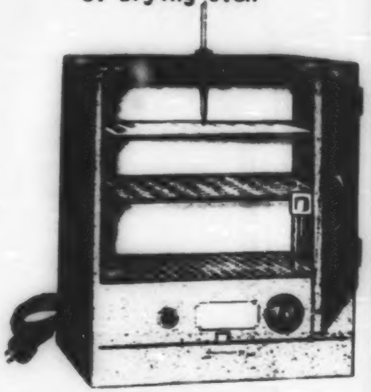
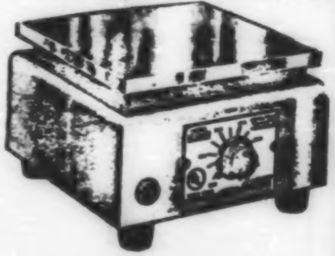
_______________ p. $Na_2SO_3 \cdot 7H_2O$

Lesson 5. Care and Use of Laboratory Equipment

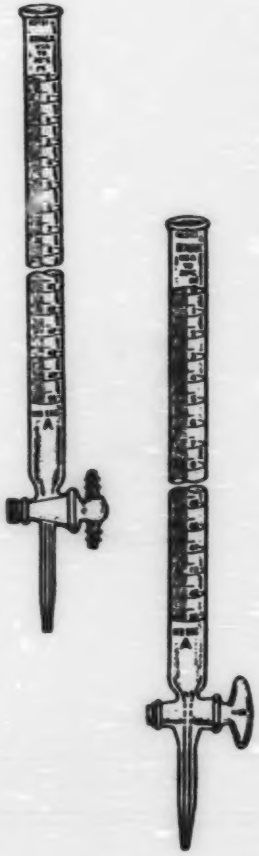
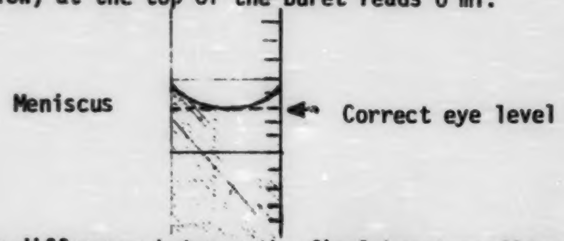
The chemical laboratory consists of a variety of equipment. In order to perform the analysis, it is necessary that one is familiar with all pertinent glassware as well as the major equipment. The following listing of items, with the correct procedures for handling are considered to be minimum. The balance will be discussed in a later chapter.

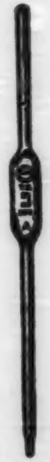
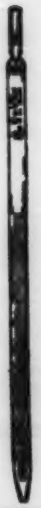
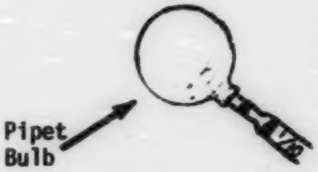
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Laboratory Equipment 1. Vacuum Systems (a) Pump 	1. Check oil level in pump.	1a. Oil level should be in glass bulb.	
	2. Connect pump to appropriate apparatus such as a filter flask.	2a. Use thick walled vacuum tubing.	
	3. Plug into 110 outlet and turn switch to ('ON') position.	3a. If the pump does not have a built in trap, then one should be placed between the pump and flask.  To Vacuum Pump Glasstubing To Filter Flask --- Glass Bottle Water Trap	
		3b. The trap will protect the pump parts from water or other chemical contaminates.	
		3c. House vacuum systems will be available at the laboratory bench. This is controlled from a central vacuum pump with a built-in water trap.	

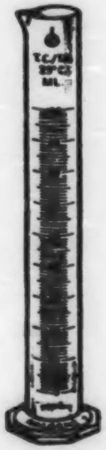
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
(b) Aspirator 	1. The aspirator is connected directly to the cold water tap.		
	2. Connect aspirator to appropriate apparatus such as a filter flask.	2a. Use thick walled vacuum tubing. 2b. [illegible] water trap, shown on previous page, section [illegible], should also be used with an aspirator since the water from the tap may back up in the filtering apparatus.	
2. Laboratory Burner  Hottest part Reducing zone Unburned gas Barrel Gas Base	1. Connect burner to a source of natural gas.	1a. If commercial gas lines are not available, self-contained gas cylinders (propane, butane, etc.) may be used.	
	2. Adjust the collar so that a small amount of air enters the barrel.		
	3. Turn the gas supply on.		
	4. Open the needle valve on the burner about 1 turn.	4a. If the gas flow is insufficient, the burner will "backfire".	
	5. Light the flame.	5a. A flint striker is recommended.	
	6. Adjust the flame so that the three zones are present.	6a. The hottest part of the flame is directly above the top of the reducing zone.	

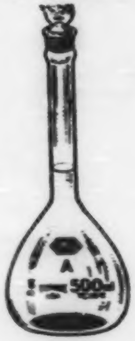
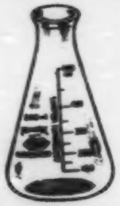
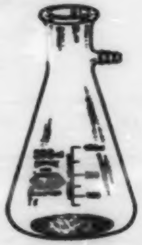
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
3. Drying Oven 	1. Plug into 110 volt outlet.	1a. The drying oven is used to dry crucibles, glassware, as well as various chemicals. It should have a heating capacity of up to 250°C and be controllable to ± 0.5°C.	
	2. Adjust control on front to desired temperature.	2a. Usually between 4 and 5 will give a temperature of from 100 - 115°C. Each oven should be calibrated to the specific temperature that is required.	
4. Hotplate and Stirrer 	1. Plug into 110 volt outlet.	1a. Since many solutions need to be heated, either a hotplate or bunsen burner may be used.	
	2. Place container to be heated or mixed on the unit.		
	3. Turn the appropriate switch 'ON'.	3a. The heating and stirring units should be able to be operated independently or together.	
5. Water Still 	1. Since all water stills operate differently, the individual instructions accompanying the still should be followed.	1a. Distilled water is required in nearly every test performed. High quality distilled water can be obtained from several commercial models. The capacity of a still should be determined by specific needs of your lab. Usually 1-2 gallons per hour is sufficient for most laboratories. It is also possible to add an ion exchange system to the water still in order to produce "ultra pure" water.	

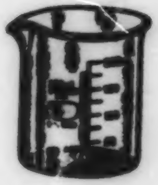
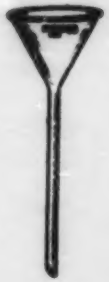
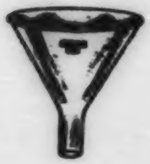
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
5. Water Still (continued)		1a. If the number of analyses to be performed in a laboratory is relatively small, the distilled water may be purchased from a commercial laboratory. It should also be noted that a water still need to be cleaned regularly. Consult the instruction manual for specific details.	
6. Desiccator	1. Remove lid.	1a. The desiccator is used for the storage of chemicals or equipment that must be kept moisture free. It contains an anhydrous material at the bottom such as Drierite®. The container may be either metal or glass. 1b. Glass desiccators should be enclosed in metal cages.	
	2. Add the Drierite® to the bottom.	2a. Drierite® is a commercial name for calcium sulfate ($CaSO_4$). Other anhydrous materials such as silica gel may also be used.	
	3. Place the porous plate in the container.		
	4. Grease the top of the container and the bottom of the lid.	4a. Commercially available silicone stopcock grease is satisfactory.	
	5. Replace the lid, making sure that it is air tight.	5a. If indicating Drierite® is used, it will change from blue to red when it has absorbed its maximum amount of moisture. This particular type can be regenerated by placing it in an oven at 200°C untill the color changes back to blue. 5b. If hot crucibles or other hot items are being cooled, leave the lid "cracked" open for about 5 minutes before sealing it airtight. This will prevent implosions.	

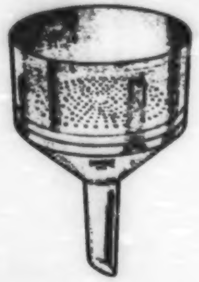
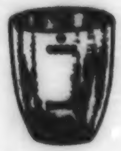
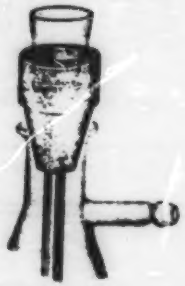
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Buret 	1. The buret is designed to deliver liquids in a controlled fashion such that additions can be made dropwise and final volume delivered determined. The straight bore, 50 ml capacity with 0.1 ml subdivisions and Teflon stopcock is recommended for general use. Burets with larger or smaller capacities are also available.		
	2. Fill the buret by adding the liquid with aid of a funnel to the top with the stopcock closed.	2a. It should be filled well above the 0 ml mark.	
	3. Bleed out the tip so that the orifice through the stopcock and tip are free of air bubbles.	3a. This should be continued until the meniscus (see below) at the top of the buret reads 0 ml. 	
	4. Dispense the liquid by grasping the stopcock with the left hand, leaving the right hand free to agitate the flask below.		
	5. Read the meniscus, after the required volume has been dispensed.	5a. The difference between the final buret reading and the initial buret reading will give the exact volume dispensed. 5b. By this method, it is not necessary to refill between each operation. Simply calculate the difference in buret reading as you continue to dispense the liquid. For example ml finished 20.2 ml start 11.6 ml used: 8.6	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
7. Buret (Continued)		5c. Be careful not to dispense below the 50 ml mark. Measuring (Mohr) pipets can be obtained from 0.1 ml to 25 ml with different subdivisions. They come in two types; those calibrated clear to the tip. The first type is sometimes referred to as "blow-out" pipet, since it is necessary to force the last drops out of the tip in order to deliver the measured volume. The second type is operated similar to a buret. The liquid is drawn up into the pipet and the desired volume delivered by allowing the liquid to drain out, using the meniscus as the indicator of volume delivered.	
8. Pipets  VOLUMETRIC PIPET  MOHR PIPET	1. Deflate and connect a rubber bulb to the top of the pipet.  2. Allow liquid to rise to the top. 3. Remove bulb and control volume with finger pressed lightly to the top of the pipet.	1a. The mouth is sometimes used to draw the liquid into the pipet; however, it is not recommended, since the solution might be strong acid, base or some other toxic solution. 1b. In place of a rubber bulb, a commercially available automatic bulb may be used. 1c. Deflate the bulb before connecting to the pipet so that any contaminant in the bulb is not forced into the pipet.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
8. Pipets (Continued)		Volumetric pipets are designed to give the greatest accuracy in pipets. They will deliver only one volume and range in capacity from 1 ml to 200 ml. Their operation is identical to the measuring pipets except that even though they are designed to deliver clear to the tip, they are NOT the blow-out type. They are calibrated to deliver the prescribed volume by simply touching the tip to the side of the container for a few seconds. The small drop remaining in the pipet is NOT included in the prescribed volume of the pipet and so it should remain in the pipet tip.	
9. Graduated Cylinder 		Graduated cylinders are used to measure large volumes of liquid and come in sizes from 10 ml to 2000 ml. They are calibrated "to deliver" or "to contain". That means in the first case that if the graduate is filled and the contents poured out, it will deliver the prescribed volume. The drops left behind are not included in the prescribed volume. In the second case, if the cylinder is filled to the selected mark, it contains the stated volume, but will not deliver this volume when poured out. Plastic cylinders are calibrated "to deliver and to contain" because water will not adhere to plastic as it does to glass. Graduated cylinders do not have the accuracy of the volumetric flask.	

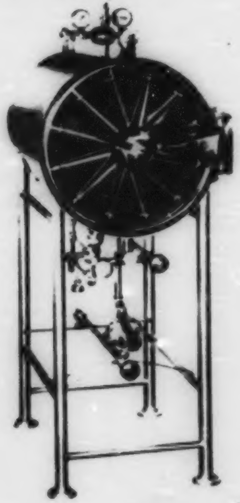
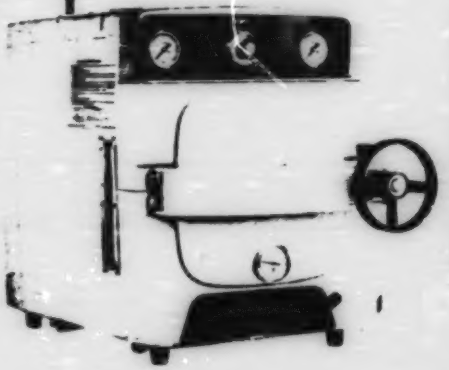
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
10. Volumetric Flask  		The volumetric flask is designed to accurately measure large volumes of liquid, primarily in the preparation of reagents and standard solutions. They range in size from 1 ml to 2000 ml. This flask is designed "to contain" and not "deliver". The last few drops of distilled water added to a volumetric flask to bring the level up to the indicating line should be added from a distilled water wash bottle.	
11. Erlenmeyer Flask 		The Erlenmeyer flask is a general purpose flask used for containing and mixing solutions. They range in capacity from 10 ml to several liters. Although they have graduations on them, they are not considered volumetric glassware and should not be used for accurate measurements of volume. The accuracy of the graduations is usually ± 5%.	
12. Filter (Side Arm) Flask 		The filter flask is essentially an Erlenmeyer flask with a side arm attachment to receive a vacuum hose. Filtration is accomplished by placing a filter funnel in the neck of the flask and drawing the liquid through with the aid of the vacuum. NOTE: Filter flasks may implode if the glass has a weak spot or the vacuum is too strong. Working behind a safety shield is suggested.	

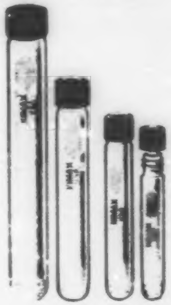
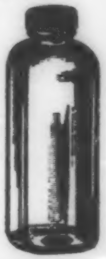
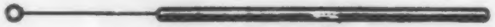
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
13. Beaker 		Beakers are non-volumetric pieces of glassware and range in size from 1 ml to 4000 ml. Although they have graduations, they should not be used to measure accurate volumes.	
14. Funnels 		The chemical filtering funnel is available in glass or plastic. It comes in a variety of sizes. They are used for accurately transferring chemical solutions.	
		The powder funnel is plastic and is used for transferring only solid chemicals. It also comes in a variety of sizes.	

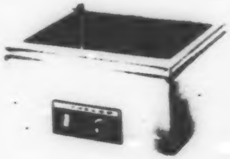
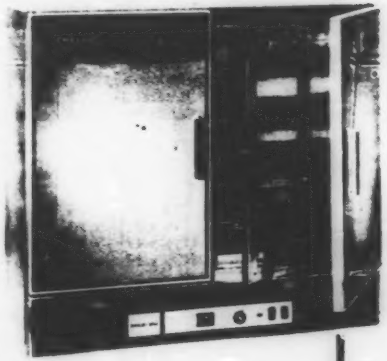
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
14. Funnels (Continued) 		The Buchner funnel, usually made of porcelain but available in plastic, is used in solids determinations. It is used in conjunction with the filter flask.	
15. Gooch Crucible 		The Gooch Crucible is used in solids determinations. A piece of glass fiber filter paper is placed in the crucible, as a filter media, prior to a suspended solids determination.	
16. Walter's Adapter 		As shown, the Walter's Adapter is used in the filter flask to hold the Gooch Crucible.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
17. Reagent Storage Bottles 		Glass stoppered reagent bottles or plastic (screw cap) bottles are available for storing prepared chemical reagents. Strong bases such as sodium hydroxide (NaOH) tend to freeze glass stoppers. Rubber or plastic stoppers may be substituted in this case.	
18. BOD Bottles 		BOD (Biochemical Oxygen Demand) bottles are specifically designed for the BOD test and have a capacity of 300 ml. They have matching ground glass stoppers and are numbered for easy identification.	

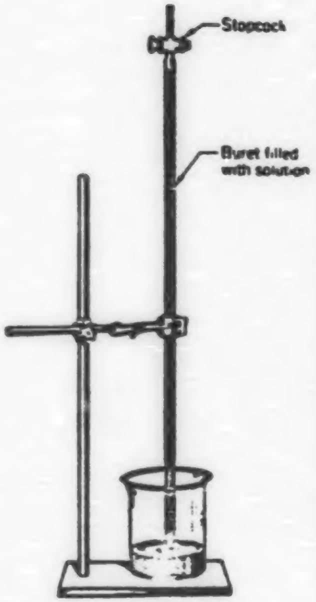
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
19. Squeeze Bottle 		Plastic squeeze (wash) bottles are used to dispense distilled water during rinsing operations.	
20. Tongs 		Crucible tongs are shown and should always be used in handling the Gooch Crucibles. There are other types of tongs available such as beaker tongs and evaporating dish tongs.	
21. Double Buret Clamp 		This clamp is designed to securely hold two burets.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
22. Utility Clamp 		The utility or combination clamp is used for thermometers, pipets, glass tubing and a variety of other laboratory glassware.	
23. Autoclave  		The autoclave is used for sterilizing solutions, bacterial growth media, and glassware. It must have the capacity to develop and hold 15 psi at 121°C for any length of time. Size is not important as long as it is large enough to accomodate the volume of work required. Bench top sterilizers are satisfactory as long as they meet the above requirements. Each autoclave is slightly different. Operating instructions are included with the instrument and should be read prior to operation. Preferably, do not operate without the instruction of someone familiar with the operation of your particular model. Use caution since the autoclave develops high pressure and high temperatures. Always remove hot items with tong or gloves.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
24. Tubes, Culture 		These tubes are available in a variety of sizes with either screw caps or snap caps.	
25. Bottles 		Dilution bottles are 125 ml volume glass bottles with one calibration at 99 ml. They are used for bacterial and sewage dilutions and can be autoclaved.	
26. Inoculation Loop 		A platinum wire loop with a 3 mm loop is used for bacteriological transfers. A wooden or aluminum handle is acceptable. The loop is sterilized between transfers by heating it to a redglow in a bunsen burner flame.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
27. Water Bath 		A water bath at 44.5°C ± 0.2°C is required for the fecal coliform test. The bath must be large enough to accomodate several plastic bags containing membrane filter dishes or tubes. This bath should have a sensitivity of at least ± 0.2°C and a range from room temperature to 100°C. Several other tests require water baths at different temperatures. Often the same bath can be used, but it must be easily adjusted between tests.	
28. Bench Model Incubator 		Most bacterial tests are run at 35°C. Therefore, an additional incubator is needed. The incubator should be large enough to accomodate the maximum number of plates which would ever be handled at the same time. Sensitivity should be at least ± 0.5°C.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Glassware Cleaning			
1. Cleaning Solution Preparation Chromic Acid	1. Weigh out 60 grams of potassium dichromate ($K_2Cr_2O_7$) in a 1 liter Erlenmeyer flask.	1a. Chromic acid cleaning solution is commercially available from several chemical supply houses. 1b. A balance that is accurate to the nearest gram is sufficient.	
	2. Dissolve the dichromate in approximately 600 ml of hot distilled water.		
	3. After the solution is cool, slowly add 400 ml of concentrated sulfuric acid (H_2SO_4) to the solution.	3a. CAUTION: Pour the acid slowly, since a large amount of heat will be generated.	
	4. After the solution is cool, transfer to a 1 liter glass stoppered storage bottle.	4a. For a stronger solution, it is permissible to decrease the volume of water used in step 2 and increase the volume of acid used in step 3 to maintain a total of 1 liter.	
2. Laboratory Detergent		Alconox® (available in powder form) or a suitable substitute should also be on hand for cleaning laboratory glassware.	
3. Cleaning Procedure for Burets		A piece of volumetric glassware is sufficiently clean if its surface is uniformly wetted by distilled water. Oily contamination prevents glass walls from being uniformly wetted; drainage is then uneven and delivery is not precise. A general rule is to clean glassware immediately after use, since it is much more difficult to remove chemicals that have been allowed to cake and age.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Glassware Cleaning (Continued)		If after cleaning a specific piece of glassware with detergent, it is found that it contains water drops upon rinsing with distilled water, then the following procedure must be followed:	
	1. Pour 100 ml of cleaning solution into a 150 ml beaker.		
	2. Invert the buret in the buret clamp and place into cleaning solution as shown in the diagram on the left.	2a. The buret should be immersed nearly to the bottom of the beaker.	
	3. Open the stopcock and apply suction with a rubber bulb drawing solution up into the buret. Fill almost to stopcock.	3a. Cleaning solution should not come in contact with the stopcock.	
	4. Close the stopcock and allow solution to stand for ten minutes.		
	5. Open stopcock to allow cleaning solution to drain back into the beaker.		
	6. Rinse the buret thoroughly with distilled water.		
	7. Remove the stopcock.	7a. If it is a ground glass type, remove grease with a paper towel.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Glassware Cleaning (Continued)			
	8. Immerse the bottom part of buret into the cleaning solution and let stand a few minutes.		
	9. Rinse thoroughly with distilled water.		
	10. Wipe the outside of the buret with a paper towel.		
	11. Replace stopcock and fill with distilled water.	11a. If the stopcock is ground glass, regrease it with silicone grease and replace. Grease should only be applied to areas indicated by the arrows. 11b. Do not grease a Teflon stopcock.	
	12. Pour the cleaning solution back into the stock bottle.	12a. The cleaning solution may be reused until it turns green, at which time it should be discarded.	
4. Pipets		Pipets may be cleaned using a similar procedure as with burets. Beakers, flasks, etc., may be cleaned by adding a small amount of cleaning solution, coating the walls of the container. The cleaning solution is then transferred back to the storage container and the item of glassware rinsed several times with distilled water. It cannot be overemphasized that proper glassware cleaning, as well as rinsing, must be carried out at all times. Trace amounts of contaminants that remain due to carelessness may seriously affect concentration or organism growth and consequently, your results. It has been found that as many as 15 rinses are insufficient to remove the contamination due to chromium.	

Lesson 6. Matter

All objects in the universe are composed of matter. Matter is anything that occupies space and has mass. The property of occupying space is easily perceived by our senses of sight and feeling. The property of mass pertains to the quantity of matter that a body contains. The mass of a certain body of matter does not change. On the other hand, the weight of a body pertains to the force of attraction of the earth for the body and is dependent upon its distance from the earth's center. If a book were taken to the top of a mountain, it would weigh less than it does at sea level. If it were taken far out into space, its weight would become neglible. In both cases the mass of the book has remained constant. When working in the laboratory we measure quantities of matter in terms of mass rather than weight because the mass of a body remains constant whereas the weight of a body is an "accident of its environment." The balance is used for measuring the mass of an object.

Physical States of Matter

Matter can exist in three states, designated as solid, liquid, and gas. These may be distinguished by certain qualtities.

a. Solids - Have a definite shape and a definite volume
b. Liquids - Occupy a definite volume but take the shape of their containers.
c. Gases - Have neither a definite shape nor a definite volume.

The physical state of a substance depends upon temperature; if the substance is a gas, then pressure is also a factor. For example, water, a liquid at ordinary temperatures, changes to a gas (steam) when it is boiled and to a solid (ice) when it is frozen. Carbon dioxide (CO_2) gas on the other hand can be changed to a liquid or a solid (Dry Ice) by cooling and compressing it sufficiently. Other gases behave similarly. Some substances may change spontaneously from one state to another. Water evaporating would be a typical example. If a substance were to evaporate very rapidly, such as ether, then it would be referred to as volatile.

The physical states of matter may be explained by assuming that matter is composed of very small particles called molecules, which are in constant motion. In solids, these molecules are packed very close together. In liquids, they are not so close together and can roll easily over one another. In gases, the molecules are widely separated and uniformly distributed throughout the container.

Properties of Matter

A property is a characteristic of a substance which enables us to recognize it. The properties of substances may be classed as either physical or chemical.

a. Physical properties include state, color, odor, taste, density, solubility, boiling point and freezing point. They are determined without changing the chemical composition of the substance.

b. Chemical properties are properties that concern the manner in which one substance reacts with another substance. The fact that silver will tarnish, or that iron will rust is a chemical property.

Changes of Matter

Matter may undergo either a physical or a chemical change.

a. A physical change is a change in state without a change in composition.

Melting of ice, freezing of water, conversion of water to steam, condensation of steam to water, melting sulfur, chopping wood, dissolving sugar in water. These are all examples of physical change. In each of these there is a change in properties but there is no alteration of the chemical composition of the substances involved.

b. A chemical change is one in which a substance loses the properties by which we recognize it, and produces a new substance. A chemical change is therefore a change in composition. When carbon, a black substance, burns in air, an invisible gas consisting of both carbon and oxygen (carbon dioxide, CO_2), is formed. When milk sours, the sugar in the milk is converted into an acid and the composition and the properties of the acid differ greatly from those of the sugar. Iron rust formed by the corrosion of iron metal contains oxygen as well as iron, and it is therefore a different substance with different properties.

Laboratory Significance

Some of the more important physical and chemical properties that are directly applicable to laboratory work are as follows:

1. The volume of a substance can change with temperature, consequently all solutions must

be returned to room temperature before dilution. Also the volumetric glassware such as buret, pipet and volumetric flask should never be heated since the graduations will change.

2. A chemical label with the words "deliquescent" or "hygroscopic" indicates that the container should always be kept tightly closed to prevent entry of water in the atmosphere.

Hygroscopic substances are substances that can absorb moisture from the air without becoming wet. Rice is placed in a salt shaker to keep the salt dry.

Deliquescent substances, when exposed to the air, absorb enough moisture to become wet. Sodium hydroxide ($NaOH$), calcium chloride ($CaCl_2$) are examples of deliquescent substances.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Volume/Temperature Dependence of Liquids	1. The following materials are needed for this experiment: a. 3 250 ml (or 200 ml) volumetric flasks. b. Bunsen burner. c. 2 600 ml beakers d. 1 metal trough e. Ice f. 1 ring and ring stand g. 1 thermometer		
	2. Fill a 600 ml beaker with 300 ml of tap water and cool in an ice bath.		
	3. Fill the other 600 ml beaker with 300 ml of tap water and heat over a bunsen burner to 85°C.	3a. Use the thermometer to determine the temperature.	
	4. Fill each volumetric flask to the base of the neck.	4a. 1 flask with cold water (from ice bath). 4b. 1 flask with hot water. 4c. 1 flask with room temperature water.	
	5. Use a squeeze bottle to fill to the graduation line.		
	6. Let all flasks come to room temperature and observe the level of the water in the flask.		
	7. Record all results in your laboratory notebook.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Deliquescent Substances	1. Place 4 sodium hydroxide (NaOH) pellets on a watch glass. 2. After the sodium hydroxide (NaOH) has been left out for six hours, note the appearance. 3. Record all results in your laboratory notebook.	1a. Note appearance immediately and mark observations in notebook.	

Lesson 7. Solutions

Wastewater is a complex combination of water, floating and settleable solids, and dissolved solids. It is possible to separate the components of wastewater by physical and mechanical processes such as screening, settling, filtration and evaporation. Since this is the case, the chemist calls wastewater a mixture.

Let us take a sample of raw wastewater and run it through a very fine filter. All the floating and settleable solids will be removed. The filter also removes the turbidity. The filtrate, the liquid which comes through the filter, is a part of the original mixture. It contains water and dissolved solids. This clear liquid could be separated into two more components by distilling off the water. The dissolved solids would be left behind. Thus, the filtrate, too, is a mixture. But it is a very special mixture called a solution. The term solution refers to a homogeneous mixture of two or more substances. The molecules of these substances are evenly distributed among one another. Because the particles in a solution are all the size of molecules, we can not see any one component. A solution appears to be one pure substance. The components of a solution will not separate by settling.

The subject of solutions has been introduced by looking at wastewater because it is a mixture known to most of you. However, there are many other solutions which are familar to you. We will now use some common solutions to continue our study of this important topic.

Chemists classify solutions into three major groups:

1. Gaseous solutions
2. Liquid solutions
3. Solid solutions

We will look at each group separately.

Gaseous solutions are made by mixing one gas in another. Air is a gaseous solution. Air is made of nitrogen, oxygen, argon, carbon dioxide and very small amounts of other gases. The molecules of each gas mix evenly to make a homogeneous mixture called air. The molecules of carbon dioxide are heavier than the molecules of the other gases but they do not settle out. We know that the amount of oxygen in a sample of air can change. There is less oxygen in a sample from the top of a high mountain than there is in a sample taken at sea level. Therefore, we must add to our description of a solution this fact:

The composition of a solution is changeable.

Liquid solutions are made by dissolving a gas, liquid or a solid in a liquid. Tap water is a solution which contains dissolved oxygen. The oxygen molecules are mixed uniformly with the water molecules to make a homogeneous mixture. The oxygen molecules do not settle out if the mixture is allowed to stand undisturbed. "Old Granddad" is an example of a liquid dissolved in another liquid. The alcohol molecules are dissolved uniformly in the water. We know this because every jigger tastes the same. The components of "Old Granddad" do not separate by settling. A sugar-water solution is an example of a solid dissolved in a liquid. The sugar crystals breakup into molecules which mix uniformly with the water molecules. This gives a mixture which is homogeneous and there is no settling. We must note here that liquid solutions also have variable compositions. Alcohol-water solutions have different strengths. Sugar-water solutions can be very sweet and not so sweet depending on the amount of sugar added.

Solid solutions are solids in which the molecules of one component are randomly mixed with the molecules of another component. An example of a solid solution is brass, an alloy of zinc and copper. Sterling silver is a solution of copper and silver.

We can now list the characteristics which are common to all solutions:

1. Each component is broken down into molecules or atoms.
2. The molecules or atoms of each component are mixed uniformly.
3. No one component will settle out.
4. Solutions are clear and transparent.
5. The composition of a solution can vary.

To complete our study of the nature of solutions we must note two properties of solutions. These properties apply to all solutions but in varying degrees. The first is the effect of mixing two substances on the total volume of the solution. When one liter of alcohol and one liter of water are mixed, the total volume is less than two liters. When sugar is dissolved in water, the volume of solution is larger than the original volume of water. Thus mixing two substances to make a solution may cause the total volume of solution to be greater or less than the total volume of liquid(s) used.

The second property is a temperature change caused by mixing two different substances. When sulfuric acid, H_2SO_4, or sodium hydroxide NaOH, are dissolved in water, the solution initially becomes hot enough to boil or at least form steam. Making solutions of either H_2SO_4 or NaOH should be done slowly and carefully. Use about half the water required and add the acid or base to the water slowly. Allow time for this mixture to cool. Then add the remaining water required slowly. Most acids and bases will cause a temperature increase when mixed with water. The temperature increase results from the reaction of the water with the acid or base. Acids react with water to produce electrically charged hydrogen atoms called hydrogen ions, H^+. Bases react with water producing hydroxide ions, OH^-. These two different reactions both produce heat.

The temperature sometimes decreases when making a solution. When sodium thiosulfate, ($Na_2S_2O_3 \cdot 5H_2O$), is added to water, the solution is initially cold. When you discover this problem in making a solution you must first dissolve the chemical in about half the required water. Allow time for warming. Then add the remaining required water.

Before we go to a new topic, three new terms must be introduced:

a. Solute
b. Solvent
c. Solubility

The solute is the substance which dissolves. The solvent is the substance which does the dissolving. For a solution involving a solid mixed with a liquid, the solid is considered the solute and the liquid is the solvent. When a liquid is mixed with water, the water is the solvent and the other liquid is the solute.

Examples:

1. A salt-water solution.	Solute-salt Solvent-water
2. An alcohol-water solution.	Solute-alcohol Solvent-water
3. An acid-water solution.	Solute-acid Solvent-water

Solubility is a term which describes the maximum amount of solute which will dissolve in the solvent. Solubility is a property of the solute not the solvent. Table salt, (NaCl), will dissolve in water. The maximum amount is 31.1 g. in every 100 g. of water. If the solvent is alcohol only 0.051 g. of NaCl will dissolve in 100 g. of solvent. When gasoline is the solvent the solubility of sodium chloride is 0.000 g. per 100 g. of solvent. You can see that the solubility of a solute will change when the solvent is changed.

The solubility of a solute in a specific solvent can be affected by temperature changes. In general, the solubility of solids increases with an increase in the temperature of the solvent.

The solubility of sodium nitrate, $NaNO_3$, in water is 75 g. per 100 g. of water at 0°C, and 127 g. per 100 g. of water at 60°C.

The solubility of gases decreases with an increase in the temperature of the solvent. The solubility of oxygen in water is about 15 mg. in 1 liter of water at 0°C, and about 9 mg. in 1 liter of water at 20°C.

The subject of solubility and the variable nature of the solubility of a solute suggests the problems of specifying the actual amount of solute dissolved in a solvent. The problem is particularly important since many chemicals must be dissolved in water before they can be used. To solve the problem chemists have developed a number called the "concentration" of the solution. The concentration number describes the amount of solute in a convenient volume of solution. Suppose 1 liter of solution contains 100 g. of potassium iodide. The concentration is 100 g. per liter of solution or simple 100 g/l of potassium iodide in water. If five liters of solution contains 750 g. of salt, then the concentration is 750 g. per 5 liters. Since 5 liters is not a "convenient" volume, we use a proportion to find that 750 g. per 5 liters is the same as 150 g. per 1 liter. We say the concentration is 150 g/l even though there are actually 5 liters of solution.

The concentration of a solution can be found directly using the formula below:

$$\text{Concentration} = \frac{\text{weight of solute}}{\text{volume of solution}}$$

For example, 600 mg. of NaCl is dissolved in 0.5 l of solution. The concentration is:

$$\text{Concentration} = \frac{600\text{ mg.}}{0.5\text{ l}}$$

Now we simplify the concentration number by dividing the denominator and the numerator by 0.5:

$$\text{Concentration} = \frac{1200\text{ mg.}}{1\text{ liter}} \quad \text{or } 1200\text{ mg/liter}$$

The concentration is normally reported in the units mg/l, g/l or ppm. If the weight and volume data are given in units other than milligrams or grams and liters, you can change the given units by the appropriate conversion factors. Then use the formula given. Remember that:

1 mg/l = 1 ppm
1000 mg/l = 1 g/l

Two other units of concentration commonly used in chemistry are normality (N) and molarity (M). These are examples of the two units:

$0.25N\ H_2SO_4$ -- means a 0.25 normal solution of sulfuric acid.

2 M NaOH -- means a 2 molar solution of sodium hydroxide.

It should be noted that the term N/50 means $\frac{1}{50}$ N or 0.02N. Also:

N/40 means $\frac{1}{40}$ N = 0.025N

N/10 means $\frac{1}{10}$ N = 0.1N

These terms are sometimes used and their format should be noted carefully.

Lesson 8. Use of Laboratory Balances

There are four types of balances commonly used in laboratory work. They are:

1) **the double-pan analytical balance,**
2) the single-pan electric analytical balance,
3) the triple beam or "trip" balance, and
4) the top-loading electric balance.

In this lesson you will receive background information and operational procedures for each of the four balances.

The double-pan analytical balance is a precision instrument used to obtain weights to the nearest 0.0001 g. This balance can weigh the oil from your skin left by a fingerprint. The maximum capacity is generally 200 g. It is a very delicate instrument and must be handled very carefully. For the best possible operation the double-pan analytical balance should be isolated from the rest of the lab in a constant temperature, constant humidity room. If this is not possible, good results can be obtained by placing the balance in a protected area of the lab away from wind currents and corrosive fumes. The balance should be positioned on a separate table with no other equipment and the table must be level and very sturdy. Never jostle this balance. The balance must be kept clean at all times to obtain good results. Operation of the double-pan analytical balance requires a set of standard weights. These weights must be kept clean and dry at all times. Never pick the weights up with your hands. Handle them only with the clean, dry forceps provided.

There are several different brands of double-pan analytical balances on the market and in use today. For this reason the procedure presented in this lesson is general in content. You will find several references in the procedure to the O & M manual for the specific balance. If you do not have an O & M manual for your balance, make a written request for one to the manufacturer. For your work in this course, your instructor will carefully describe all the controls and operations of the balance you will use.

The single-pan electric analytical balance is also accurate to 0.0001 g. The maximum capacity is generally 200 g. It is very delicate and requires all the precautions stated for the double-pan analytical balance. This balance is a self-contained unit and requires no standard weight set.

Single-pan analytical balances are produced by several manufacturers and each manufacturer makes many different types of balances. Therefore, the procedure given in this lesson is very general. You must have an O & M manual for your balance.

The manufacturer will provide one upon request. The instruction you receive in this lesson will allow you to operate any single-pan analytical balance after a little practice. The instructor will fully explain the balance you will use.

The triple beam balance is one of the least accurate balances used in laboratory work. It weighs to the nearest 0.1 g. You may have one that weighs to the nearest 0.01 g. The capacity of this balance ranges from 200 g. to 610 g. The trip balance is very sensitive to air currents in the lab. A person walking by the balance while it is in operation can cause inaccurate results. The trip balance is a delicate instrument. It requires all the care of a double-pan analytical balance. Since the trip balance is not self-enclosed, use it only in an isolated area of the lab where the air currents are at a minimum.

The operational procedure for the trip balance is very general. It attempts to include all possible steps in the operation of a trip balance.

The top-loading electric balance is the last type of common laboratory balance. It weighs to the nearest 0.1 g., 0.01 g., or 0.001 g. depending on the style you purchase. The total capacity of these balances ranges from 170 g. to 11,000 g. High capacity instruments have low accuracy and low capacity instruments have high accuracy. The unit is self-contained requiring no weight set.

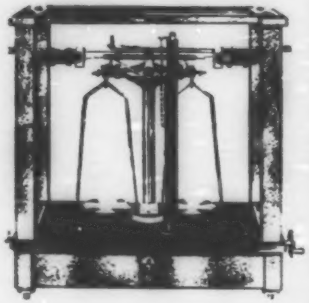
The top-loading balance is a delicate instrument. It is very sensitive to air currents. Place this balance in an isolated area of the lab on a sturdy table. Keep it clean at all times.

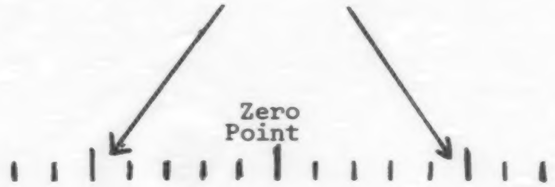
The introduction which you have just read includes several pieces of information of the types of balances you will need to use in the laboratory. In general, all balances are delicate instruments. They all require careful handling. You may use one table in the lab to hold all of your balances. Remember to choose an isolated area.

The following is a list of do's and don't's which apply to all balances.

1) HANDLE ALL CONTROLS CAREFULLY AND SLOWLY.
2) NEVER PUT CHEMICALS DIRECTLY ON THE BALANCE PAN. USE A WEIGHING BOAT OR BEAKER.
3) NEVER WEIGH AN OBJECT WHILE IT IS HOT, OR EVEN WARM.

DOUBLE-PAN BALANCE

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Balance Inspection	1. Dust the pans with a soft hair brush.	1a. The pans must be clean and dry.	
	2. Slowly operate all balance control knobs, levers, etc.	2a. All controls must operate freely.	
	3. Return all controls to ready position.	3a. Rider to zero. 3b. Zero on milligram strip must match zero on vernier scale. 3c. Beam arrest knob to arrest position. 3d. Pan arrest knob to arrest position.	
	4. Inspect the beam and pan support knife edges.	4a. The knife edges must be the proper position for correct operation.	
	5. Release the beam arrest.		
	6. Release the pan arrest mechanism.	6a. The beam should swing freely from side to side.	
	7. Return arrest knobs arrest position.		
	8. Inspect level indicator(s)	8a. If balance is not level, adjust the movable legs to level the balance.	
2. Determine the Zero Point	1. With the balance door closed, release the beam arrest and pan arrest mechanisms.	1a. The pointer should oscillate from 3 to 5 small divisions on either side of the center of the reference scale.	
	2. Count the number of small divisions between the right most point and left most point of the swing.	2a. Count to the nearest half division.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation (Continued)	3. Divide this number by 2.	3a. The number should be to the nearest half.	
	4. From the original left most point, count to the right the number of divisions equal to the number you have calculated.	4a. This is the midpoint of the swing which is called the zero point. 4b. The zero point must be determined before each weighing procedure.	
	5. Return the beam and pan arrests to the ready position.	 Total swing - 7 1/2 divisions 7 1/2 ÷ 2 = 3 3/4 or 3 1/2 to the nearest half.	
B. Weighing an Object			
1. Determine the weight to the nearest whole gram.	1. Place the object to be weighed on the left pan.	1a. The balance door is left open during this part of the procedure.	
NOTE: FOR CONVENIENCE, MAKE A PERMANENT RECORD OF EQUIPMENT WHICH IS USED FREQUENTLY AND THE WEIGHT OF EACH ITEM TO THE NEAREST ± .1 g.	2. Approximate the weight and add this amount to the right pan.	2a. Use forceps to transfer the weights from the box to the pan. 2b. Handling weights causes the metal to corrode and changes the weights of the standards.	
	3. Release the beam arrest slowly.	3a. Releasing too fast may cause the beam knife edge to jump off its support.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	4. Release the pan arrest slowly.	4a. Releasing too fast may cause the pan supports to jump off their knife edge. 4b. If the pointer swings to the left of the zero point and remains there, too much weight is on the right pan. 4c. If the pointer swings to the right of the zero point and remains there, not enough weight is on the right pan.	
	5. Return the beam and pan arrests to the ready position.	5a. Never add or subtract weight from the left or right pans with the beam and pan arrests released.	
	6. Add or subtract weight from the right pan as indicated by the results of Step 4.		
	7. Slowly release the beam	7a. If the pointer swings mostly to the left of the zero point and finally remains on the left, there is too much weight on the right pan. 7b. If the pointer swings most to the right of the zero point and finally remains on the right, there is not enough weight on the right pan.	
	8. Return the beam and pan arrests to the ready position.		
	9. Repeat steps 6, 7, and 8, adding or subtracting smaller amounts of weight.	9a. Repeat until adding one gram of weight causes the pointer to come to rest on the left of the zero point.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	10. Remove the one gram of weight from the right pan.	10a. This is the one gram of weight mentioned in 9a.	
	11. Find the total weight on the right pan and record on your benchsheet.	11a. This is the weight to the nearest gram.	
2. Determine the weight to the nearest tenth of a gram.	1. Close the balance door.	1a. The balance door will remain closed during the balance of this procedure. 1b. The door is closed to prevent currents from affecting the freely swinging beam.	
	2. With the rider control, move the rider to the 0.5 g. position.	2a. Handle the control carefully. 2b. If the rider drops off the beam, use forceps to place it back on the beam.	
	3. Release the beam and pan arrests.	3a. If the pointer swing favors the left of the zero point, the weight shown by the rider is too much. 3b. If the pointer swing favors the right of the zero point, the weight shown by the rider is not enough.	
	4. Return the beam and pan arrests to the ready position.		
	5. Add or subtract weight with the rider as required by the results of step 3.	5a. Add or subtract weights by moving the rider in one tenth gram amounts.	
	6. Repeat Steps 3, 4, 5.	6a. Repeat, until adding one tenth of a gram with the rider causes the pointer swing to favor the left of the zero point.	
	7. Move the rider to the position which has been determined to be less than one tenth of a gram too light.	7a. The rider is in the correct position, when moving it one place to the right would be too much weight. 7b. The pointer swing will favor the right of the zero point when the rider is in its final, correct position.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	8. Record the value of the rider position as the tenths digit of your weight.		
3. Determine the weight to the nearest ten-thousandth of a gram.	1. The balance door must be closed during this procedure.		
	2. Release the beam and pan arrests.	2a. Release the pan arrest very slowly so that the pointer swings as little as possible.	
	3. Slowly add weight by rotating the knob which controls the milligram scale.	3a. Do not watch the milligram strip. Watch the pointer.	
	4. Stop adding weight when the pointer is resting on the zero point or is oscillating slightly about the zero point.		
	5. Return the pan arrest to the ready position.		
	6. Release the pan arrest.	6a. The pointer must swing freely 3 to 5 small division to the left and the right of the zero point. 6b. If the pointer swing favors the left of the zero point, there is too much weight from the gold chain. 6c. If the pointer swing favors the right of the zero point, there is not enough weight from the gold chain.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	7. Rotate the chain control knob very slightly to add or subtract weight as required by step 6 results.	7a. Adjust the weight so that the pointer is swinging evenly about the zero point. 7b. The swing should not be less than 2 small divisions to the left and right of the zero point.	
	8. Read the value on the milligram scale which is opposite the zero of the vernier scale.	8a. The hundredths and thousandth digits of the weight will be obtained from this scale. 8b. The milligram scale runs from 0 - 100 milligrams. 8c. If the zero of the vernier scale is between two small divisions on the milligram scale, record the smaller milligram value. 8d. If the zero of the vernier scale is between 0 and 10 on the milligram scale, record a zero in the hundredths position of the weight and the appropriate digit in the thousandths position.	
	9. Obtain the ten-thousandths digit of the weight from the vernier scale.	9a. Determine which division on the vernier scale most closely matches any division on the milligram scale. 9b. Record the value of this vernier scale division as the ten-thousandths digit of the weight.	
	10. Return the pan and beam arrests to the ready position.		
	11. Remove the object from the left pan.		
	12. Remove the weights from the right pan.	12a. Use forceps.	
	13. Return milligram scale to the zero position.		
	14. Return the rider to the zero position.		
	15. Close the balance door.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing a Chemical by Weighing into a Weighing Boat.			
1. Determine the weight of the boat.	1. Weigh the boat as specified in Procedure B of this EMP.	1a. Keep a permanent record of the approximate weight of the weighing boats you use.	
2. Determine the total weight for boat and chemical.	1. On the benchsheet add the weight of chemical desired to the weight of the boat.	1a. Use the benchsheet to keep a record of your mathematics.	
	2. Add weight to the right side of the beam.	2a. Use weights as necessary from the weight box.	
	3. Add chemical to the boat very slowly.	3a. If the boat must be removed from the balance pan at any time, return the pan and beam arrests to the ready position. 3b. By adding chemical to the boat, bring the pointer slowly to the zero point. 3c. Use the pan arrest to slow the swing of the pointer if necessary.	
	4. When the pointer is very near the zero point, return the pan arrest to the ready position.		
	5. Close the balance door.		
	6. Release the pan arrest.	6a. The pointer should swing freely about the zero point. 6b. If the swing favors the left of the zero point, the chemical weight is too low. 6c. If the swing favors the right of the zero point, the chemical weight is too high.	
	7. Turn the release knob to the arrest position.		
	8. Open the balance door.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing a Chemical by Weighing into a Weighing Boat.	9. Add or remove chemical to the boat as required.	7a. Only small amounts of chemical.	
	10. Close the balance door.		
	11. Release all arrests slowly.	9a. Pointer should swing at least 3 small divisions on either side of the zero point. 9b. If the pointer swings evenly about the zero point you are finished. 9c. If it does not, determine whether chemical must be added or removed as in step 6.	
	12. Repeat steps 7, 8, 9, 10, and 11, as necessary.	10a. When the correct amount of chemical is in the boat proceed to step	
	13. Return all controls to the ready position.		
	14. Open balance door and remove weighing boat with chemical.		
	15. Close balance door.		

SINGLE-PAN ANALYTICAL BALANCE

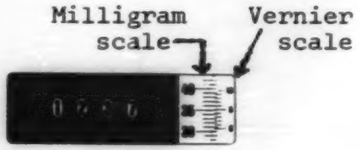
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Balance Inspection	1. Dust the pan with a soft hair brush.	1a. The pans must be clean and dry.	
	2. Operate all balance control knobs, switches, etc.	2a. All controls must operate freely. 2b. With the release knob at full release, the re light must be on the read panel.	
	3. Return all controls to the ready position.	3a. Main release knob to full arrest position. 3b. All weight control knobs at the zero position.	
	4. Level the balance.	4a. Inspect the level indicator. 4b. Adjust the movable legs to level the balance.	
2. Zero the Balance	1. Turn the release knob to full release.		
	2. With the zero control, make the readout panel show all zeros.	2a. See the O & M for the specific balance to locate the zero control. 2b. If the balance has a milligram scale and a vernier scale in the readout panel, make the zeros on both scales match.	
	3. Turn the release knob to the arrest position.		
B. Weighing an Object			
1. Preweigh the Object	1. Place the object on the pan.		
	2. Determine the weight of the object to the nearest whole gram with the automatic preweigh control.	2a. This step applies only when the balance has an automatic preweighing mechanism. (See the manufacturer's O & M manual for location of the control knob and operation instructions.) 2b. If the balance does not have an automatic preweigh feature, go to procedure 2.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	3. Using the hundreds, tens, and unit gram control knobs, set the weight on the readout panel to value given in step 2.	3a. Set weight to the nearest gram. 3b. If the preweigh value was between two whole gram values, set the weight to the smaller value. 3c. Some balances have one control knob for the hundreds and tens of grams. See the O & M manual for your balance.	
	4. Turn the release knob to full release.	4a. The readout panel will indicate that the weight on the balance is too low.	
	5. Turn the release knob to the arrest position.		
2. Determine the Weight to the Nearest Whole Gram.	1. Place the object on the balance pan.		
	2. Close the balance door.		
	3. With the hundreds of grams control add 100 g.	3a. Some balances have a combined 100 g/10 g. control. If this is the case, go to step 8.	
	4. Turn the release knob to the full release position.	4a. Some balances have a half release position. This position must be used if available. See O & M manual. 4b. The balance readout panel will indicate whether you have dialed too much weight or not enough weight. See the O & M manual for the indicator.	
	5. Turn the release knob to arrest.	5a. When weight is dialed on or off, the balance in the hundreds position, the release knob must be in the arrest position.	
	6. Add or subtract 100 g. from the balance as indicated by the readout panel.	6a. The weight is correct when an additional 100 g. would be too much weight.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	7. Repeat steps 4, 5, and 6 as necessary.		
	8. With the tens of grams control add 10 g.		
	9. Turn the release knob to the full release position.	9a. Some balances have a half release position. This position must be used if available. See O & M manual. 9b. The balance readout panel will indicate whether you have added too much weight or not enough weight. See the O & M manual for indicator.	
	10. Turn the release knob to arrest.	10a. When weight is dialed on or off the balance in the tens position the release knob must be in arrest position.	
	11. Add or subtract 10 g. from the balance as indicated by the readout panel.	11a. The weight is correct when an additional 10 g. would be too much.	
	12. Repeat steps 9, 10, and 11 as necessary.		
	13. Turn the release knob to the full release position.	13a. Do not use the half release position even if it is available.	
	14. With the one gram control, add 1 g.	14a. The balance readout panel will indicate too much weight or not enough. See the O & M manual for the indicator.	
	15. Add or subtract 1 g. of weight as indicated by the readout panel.	15a. The release knob remains at full release.	
	16. Repeat step 15 as necessary.	16a. The weight is correct when addition of 1 g. would be too much weight.	

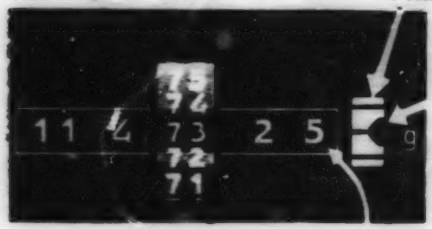
(Continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)		16a. NOTE: Some balance have a 0.1 g. control and some do not. See the O & M manual. If the balance has a 0.1 g. control, continue with procedures 3 and 4. If the balance does not have a 0.1 g. control, go to procedure 5.	
3. Determine the Weight to the Nearest One Tenth Gram. SEE NOTE IN INFORMATION COLUMN.	1. With the 0.1 g. control add 0.1 g. of weight to the balance.	1a. The balance readout panel will indicate too much weight or not enough. See O & M manual for the indicator.	
	2. Add or subtract 0.1 g. of weight to the balance.	2a. The release knob remains in the full release position.	
	3. Repeat step 2 as necessary.	3a. The weight is correct when an additional 0.1 g. would be too much.	
	4. Record the hundreds, tens, units, and tenths digits of the weight on your benchsheet.		
4. Determine the Weight to the Nearest 0.0001 g.	1. Read the value of the milligram scale across from the zero on the vernier scale.	1a. The zero of the vernier zero is the reference point for the milligram scale. 1b. If the vernier zero is between two small divisions on the milligram scale, record the value of the division with the lesser value. 1c. If the vernier zero is between 0 and 10 on the milligram scale, the hundredths digit of the weight will be 0.	



Readout Panel showing total weight of 123.7306 g.

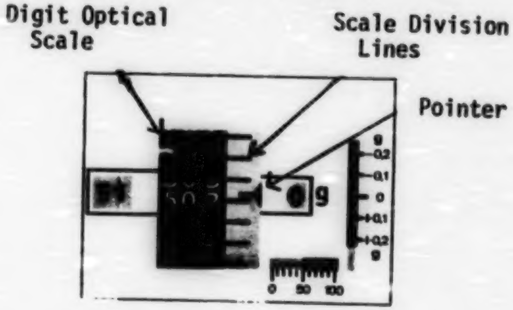
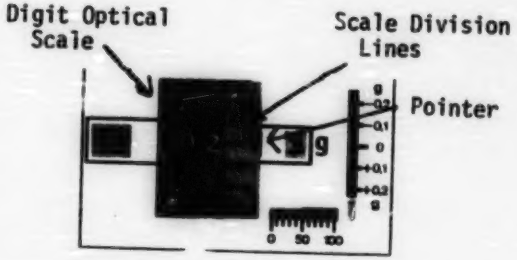
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	2. Record the hundredths and thousandths digit of the weight.	2a. On your benchsheet.	
	3. Read the vernier scale for the ten-thousandths digit of the weight.	3a. Find the vernier scale division which most closely matches any division on the milligram scale.	
	4. Record the value of vernier scale division which matches a milligram scale division.	4a. On your benchsheet.	
	5. Turn the release knob to full arrest position.		
	6. Remove the object from the balance.		
	7. Return all controls to the ready position.	7a. Turn weight controls slowly.	
5. Determine the Weight to the Nearest 0.0001 g. See Note in the Information Column.		NOTE: This procedure applies only to balances with a completely digital readout in the readout panel. These balances have no vernier scale.	

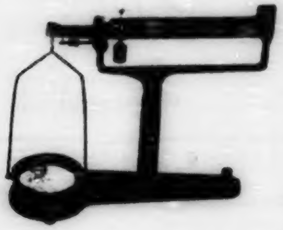
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)	1. Turn the fine adjustment control until a fine adjustment division is located precisely in the light slot of the index fork.	1a. See figure below.  Fine adjustment division Index fork 11 4 73 2 5 g 72 71 Readout Panel showing total weight of 114.7325 g.	
	2. Record the result.	2a. On your benchsheet.	
	3. Return all controls to the ready position.		
C. Weighing a Chemical by Weighing into a Weighing Boat.			
1. Determine the Weight of the Boat.	1. Use the appropriate procedures in part B.	1a. The boat will remain on the balance pan at the end of part B.	
2. Set the Weight Controls on the Balance.	1. On the benchsheet, add the weight of the boat and the desired weight of the chemical.		

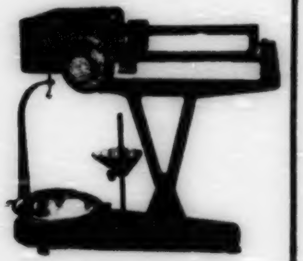
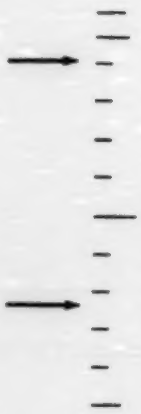
OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing a Chemical by Weighing into a Weighing Boat. (Continued)	2. Set weight control knobs to the total weights.	2a. The 100 g., 10 g., 1 g., and 0.1 g. controls. 2b. If there is no 0.0 g. control and no vernier scale, set the thousandths and ten-thousandths digit with the fine adjustment control.	
3. Weighing the Chemical.	1. Open the balance door.		
	2. Turn release knob to full release.		
	3. Slowly add chemical to the boat.	3a. Until the readout panel indicates the proper weight.	
	4. Close the balance door.		
	5. Turn release knob to full arrest.		
	6. Turn release knob to full release.		
	7. Read total weight on readout panel.	7a. If weight is not correct, go to step 8. 7b. If weight is correct, go to step 10.	
	8. Add or subtract chemical from the boat as necessary.	8a. If chemical must be removed, turn release knob to arrest before removing the chemical.	
	9. Repeat steps 4, 5, 6, 7, and 8 as necessary.	9a. Until weight reading is correct.	
	10. Turn all controls to ready position.		
	11. Remove boat and contents from pan.		
	12. Close balance door.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation			
1. Balance Inspection	1. Clean the pan with a soft cloth or towel.	1a. The pan is removable. 1b. Use soap and water if necessary. 1c. Pan must be clean and dry.	
	2. Operate all controls.	2a. All controls must operate freely.	
	3. Return all controls to the ready position.	3a. Weight control and digital counter to zero. 3b. Readout panel must show zeros in all weight readouts.	
	4. Level the balance.	4a. Center the level indicator bubble in the center of the circular marker by turning the legs at the front of the balance.	
2. Zero the Balance	1. Turn the balance on.	1a. There will be an on/off switch. See O & M manual for location.	
	2. Set the readout panel to read exactly zero.	2a. Use the zero-adjustment control. See O & M manual for location. 2b. Some balances have course and fine zero-adjustment controls. See the O & M manual.	
B. Weighing an Object	1. Place the object on the pan.	1a. Gently. 1b. If digits appear and remain on the optical scale of the readout panel, go to step 3, if not, go to the next step.	
	2. Turn the weight control knob to add weight one unit at a time.	2a. Unit weight may be 10 g., 100 g., 500 g., or 1,000 g. 2b. Add weight slowly.	

(Continued)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object (Continued)		2c. Until digits remain on optical scale.  Digit Optical Scale Scale Division Lines Pointer g 0.2 0.1 0 0.1 0.2 g 0 50 100 Readout Panel before final adjustment.	
	3. Turn the digital counter until the next lower scale division line matches the pointer. 4. Turn the balance off. 5. Remove object from pan. 6. Turn all controls to the ready position.	3a. See the O & M manual for the location of the digital counter control.  Digit Optical Scale Scale Division Lines Pointer g 0.2 0.1 0 0.1 0.2 g 0 50 100 Readout Panel showing total weight of 1150.23 g.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing a Chemical by Weighing into a Boat.	1. Determine the weight of the boat.	1a. Use procedure B.	
	2. Add the weight of boat and desired weight of chemical.	2a. Use benchsheet.	
	3. Set the first and last digits of the total on the readout panel.	3a. Set all the digits which do not appear on the optical scale. See the O & M manual.	
	4. Slowly add chemical to the boat.	4a. Until optical scale shows the correct digits and the scale division line matches the pointer.	
	5. Turn off balance.		
	6. Remove the boat and contents.		
	7. Return all controls to the ready position.		
A. Equipment Preparation			
1. Balance Inspection	1. Clean the pan with a soft cloth or towel.	1a. Pan must be clean and dry.	
	2. Place all weights and weight controls at the zero position.	2a. Balance may have only weights on the beam or weights on the beam plus a weight control dial. See the O & M manual.	
	3. Level the balance.	3a. Most triple-beam balance do not have level controls. They must be placed on a level surface.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Equipment Preparation (Continued) 1. Balance Inspection (Continued) 			
2. Determine the Zero Point of the Swing	1. Observe the range of the pointer swing.	1a. Count the total number of small divisions above and below the center division. 1b. Swing range must be 3 to 5 divisions above and below the center division.	
	2. Determine the center point of the swing range.	2a. Divide the total number of divisions by 2. Count from original uppermost point of the swing down to the center point. 2b. This is the zero point of reference for this weighing. 2c. Determine the zero point for all weighings.	
Zero point. Total swing 6 1/2 divisions	6 1/2 ÷ 2 = 3 1/4 or 3	NOTE: This type of balance can be quickly zeroed by a zero adjustment control. (See O & M Manual for location and operation.) This procedure would replace the zero point determination.	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Weighing an Object	1. Place the object on the pan.	1a. Gently.	
	2. Move the largest rider to the right one notch at a time.	2a. Until an additional notch adds too much weight. The pointer will remain below the zero point. Then, move the rider back one notch.	
	3. Repeat step 2 with smaller riders.		
	4. Move the digital slider to the right in short increments.	4a. If the balance has a dial, go to step 8. 4b. Move until the pointer swings evenly about the zero point. 4c. If the pointer swings more than 3 divisions above and below the zero point, dampen the swing by touching the pointer gently with a pencil point. 4d. The slider should be moved to the left if the pointer swing favors the area below the zero point.	
	5. Read the total weight shown by the riders and digital slider.		
	6. Return all weights to the zero position.		
	7. Remove object from pan.		
	8. Turn dial slowly.	8a. Until the pointer swings evenly about the zero point.	
	9. Total the weights on the beam and the dial.	9a. See O & M manual for reading the dial.	
	10. Return all weights and dial to the zero point.		
	11. Remove object from pan.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
C. Weighing a Chemical by Weighing into a Boat	1. Determine the weight of the weighing boat.	1a. Use procedure B.	
	2. Add the weight of the boat and the desired weight of the chemical.	2a. Use the benchsheet.	
	3. Set the total weight on the balance.	3a. Using riders and slider on dial.	
	4. Add chemical to the boat.	4a. Until the pointer swings evenly about the zero point. 4b. Pointer should not swing more than 3 small divisions above and below the zero point. 4c. Add or remove chemical until pointer indicates the correct amount. 4d. Swing mostly above zero point indicates too much chemical in boat. Swing mostly below zero point means not enough chemical in the boat.	
	5. Remove boat and contents from pan.	5a. Gently.	
	6. Return rider and slider or dial to zero position.		

Lesson 9. Volumetric Analysis

In volumetric analysis, the analyst determines the volume of a solution, the exact concentration of which is known, required for reaction with a known amount of sample to be analysed. The solution of known concentration is called the standard solution. The process used to carry out the addition of the standard solution to complete the chemical reaction is known as a titration. The volume of solution added is measured with a buret. Volumetric methods are often called titrimetric methods since they require a titration.

In a dissolved oxygen determination referred to as (D.O.) the final step in the analysis is a volumetric titration using sodium thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$) as the standard reagent. The exact concentration of the thiosulfate is determined by titration against a primary standard potassium biiodate [$KH(IO_3)_2$]. A primary standard is prepared in such a way that its concentration is known, the concentration of the thiosulfate may be determined. The concentration of these species will be referred to as "Normal" represented by N. It is important that this letter be associated with the numercial concentration at all times, i.e. 0.0375N sodium thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$). This means that the solution of sodium thiosulfate has been prepared in such a manner that its concentration is now 0.0375 Normal or 0.0375N.

Following is a procedure for the preparation and standardization of sodium thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$) using a primary standard potassium biiodate [$KH(IO_3)_2$].

Preparation and Standardization of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation			
1. Sodium Thiosulfate Stock Solution, 0.75 Normal	1. Boil about 1500 ml of distilled water for 3 minutes.		
	2. Allow the water to cool to room temperature.		
	3. Weigh 186.15 gms of sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5\ H_2O$	3a. Use an analytical balance. 3b. If one liter of solution is not needed, the amount of chemical and water may be cut by one-half, one-third, etc.	
	4. Dissolve in about 500 ml of the cooled distilled water.		
	5. Dilute to 1 liter (1) in a volumetric flask and mix thoroughly.		
	6. Transfer to a dark storage bottle.		
	7. Add 5 ml of chloroform.	7a. Use a small graduated cylinder to measure the chloroform.	
	8. Store the solution in the refrigerator.		
2. Sodium Thiosulfate Standard Titrant, 0.0375 Normal (0.0375N)	1. Dilute 25.0 ml of the sodium thiosulfate stock solution to 500 ml in a volumetric flask and mix thoroughly.	1a. Use a 25.0 ml pipet to measure the sodium thiosulfate stock solution. 1b. Be careful not to draw any chloroform into pipet.	

Preparation and Standardization of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation (Continued)	2. Add 3 ml of chloroform.	2a. Use a small graduated cylinder to measure the chloroform.	
	3. Transfer the solution to a storage bottle and label as 0.0375N (Approximate) sodium thiosulfate $Na_2S_2O_3 \cdot 5\ H_2O$		
	4. Store the solution in the refrigerator.		
3. Potassium Biiodate Standard [$KH(IO_3)2$] 0.0375N	1. Dry about 6 g. of potassium biiodate [$KH(IO_3)_2$] in the oven at 103°C for 2 hours.	1a. Use a trip balance.	
	2. Cool in a desiccator.		
	3. Weigh 2.418 g. of the biiodate.	1a. Use an analytical balance and a weighing boat.	
	4. Transfer the biiodate to a 500 ml volumetric flask, washing the weighing boat with distilled water.		
	5. Dissolve the biiodate in about 250 ml of distilled water.		
	6. Dilute to 500 ml in a volumetric flask and mix thoroughly.		

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
A. Reagent Preparation (Continued)	7. Transfer 125 ml of this solution to a clean volumetric flask.	7a. Use a 100 ml and a 25 ml pipet to measure the biiodate solution.	
	8. Dilute this solution to 500 ml and mix thoroughly.		
	9. Transfer to a storage bottle and label as 0.0375N potassium biiodate [$KH(IO_3)_2$] primary standard.		
4. Sulfuric Acid (H_2SO_4) Solution, 10% by Volume.	1. Pour 90 ml of distilled water into a 125 ml Erlenmeyer flask.	1a. Use a graduate cylinder to measure the distilled water.	
	2. Add 10 ml of concentrated sulfuric acid (H_2SO_4) to the flask and mix thoroughly.	2a. Use a small graduate cylinder to measure the sulfuric acid. 2b. CAUTION: Heat will be generated by the addition.	
	3. Allow the solution to cool to room temperature and transfer to a small glass-stoppered bottle.		
B. Standardization of Sodium Thiosulfate Titrant.			
1. Potassium Biiodate Standard [$KH(IO_3)_2$]	1. Weigh 1-3 g. of potassium iodide (KI)	1. Use a trip balance.	
	2. Transfer the potassium iodide (KI) to a 500 ml Erlenmeyer flask.		

Preparation and Standardization of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5 H_2O$)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Standardization of Sodium Thiosulfate Titrant. (Continued)	3. Dissolve in 100-150 ml of distilled water and mix thoroughly.	3a. Use a graduate cylinder to measure the water.	
	4. Add 10 ml of 10% by volume sulfuric acid solution and mix thoroughly.	4a. Use a small graduate cylinder to measure acid.	
	5. Pipet 20.0 ml of the potassium biiodate standard (0.0375N) into the solution and mix thoroughly.		
	6. Place the solution in the dark for 5 minutes.	6a. The color of the solution is reddish-brown.	
	7. Add enough distilled water to bring the volume to 300 ml and mix thoroughly.	7a. Use a graduate cylinder to measure the water.	
2. Titration	1. Fill a 50 ml buret with the standard sodium thiosulfate solution.	1a. Make sure there are no air bubbles in the tip of the buret.	
	2. Add the solution from the burette to the biiodate solution at a fast dropwise rate.	2a. Swirl the biiodate solution during the addition.	
	3. When the color changes to a pale yellow, stop the addition of sodium thiosulfate.		

Preparation and Standardization of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Standardization of Sodium Thiosulfate Titrant. (Continued)	4. Add 1-2 ml of starch solution and mix thoroughly.	4a. Use a small graduate cylinder to measure the starch solution. 4b. The color of the solution is light medium blue.	
	5. Continue the addition of sodium thiosulfate and swirling.		
	6. Stop the addition when 1 drop of sodium thiosulfate causes the solution to turn colorless.	6a. Some particles or turbidity may be present but the color of the solution should be similar to that of water.	
	7. Record the ml of sodium thiosulfate used.		
	8. Repeat the standardization at least once more and use the average titration result in the following calculation.		
3. Calculations	1. Divide the ml of sodium thiosulfate used in 0.75.	1a. The result is the normality of sodium thiosulfate. The formula used above is arrived at as follows: Normality of sodium thiosulfate x ml of sodium thiosulfate = normality of biiodate x ml of biiodate. Three of the four quantities are known: 1. ml of thiosulfate = volume used in the titration [step 7 above) 2. Normality (N) of biiodate = 0.00375 (Step 9, page 104.)	

Preparation and Standardization of Sodium Thiosulfate ($Na_2S_2O_3 \cdot 5\ H_2O$)

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
B. Standardization of Sodium Thiodulfate Titrant. (Continued)		3. ml of biiodate = 20.0 (Step 5, page 105.) Therefore: Normality of thiosulfate = $\frac{20.0\text{ ml} \times 0.0375\text{ N}}{\text{ml of thiosulfate used in titration}}$ $= \frac{0.75}{\text{ml of thiosulfate used in titration}}$ For example, if the titration required 18.5 ml of thiosulfate, then: $\frac{0.75}{18.5} = 0.0405\text{N}$ This is the correct normality of the standard thiosulfate solution and should be recorded on the thiosulfate bottle. (See step 3, page 103.)	

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
		COMMON ERRORS IN A VOLUMETRIC DETERMINATION a. Uncertainty in purity of the primary standard. b. Decomposition of the solution. c. Changes in temperature. d. Careless laboratory technique. e. Errors in weighing. f. Mechanical losses of solution during transfer of solution. g. Improper mixing of solutions. h. Dirty burets, pipets, and other similar apparatus.	

MODULE III - MICROBIOLOGY

Lesson 1. General Introduction

Biology is the science that deals with living things. Macrobiology is that area of biology which deals with those living things large enough to be seen by the naked eye, whereas microbiology involves living organisms that can only be seen with the aid of magnification.

Two major divisions of biology are botany, the science of plant life, and zoology, the science of animal life. Included under botany would be such microorganisms as algae, yeasts, and molds and such macroorganisms as fungi, grasses, and trees. Zoology would include such microorganisms as protozoa and rotifers and macroorganisms such as insects, birds, fishes and mammals.

MEASUREMENT AND MICROBIAL SIZE

1 inch = 2.54 cm
.39 inch = 1 cm = 10 mm
.039 inch = .1 cm = 1 mm = 1000µ
.0039 inch = .01 cm = .1 mm = 100µ
.00039 inch = .001 cm = .01 mm = 10µ
.000039 inch = .0001 cm = .001 mm = 1µ = 1000 nm
.0000039 inch = .00001 cm = .0001 mm = .1µ = 100 nm
.00000039 inch = .000001 cm = .00001 mm = .01µ = 10 nm
.000000039 inch = .0000001 cm = .000001 mm = .001µ = 1 nm=10Å

Protozoa - 100µ
Blood Cells - 10µ
Bacteria - 1µ
Viruses - .1µ
Molecules - .001µ
Atoms - <.001µ

µ = Micron or Micrometer
nm = Nanometer or Millimicron
Å = Angstrom = .00000001 cm

An organism is studied as to its nature, whether it is plant or animal or as an individual. Studying an organism as an individual includes looking at its morphology which entails anatomy, histology, and cytology; and its physiology and embryology. Organisms can be studied as groups either taxonomically, ecologically or genetically.

WHAT IS "LIFE?"

A definition of "living" is rather difficult to make. It is more easily described by a series of special attributes. An understanding of these attributes is critical but not definitive in every instance. The basic attribute of all living things is organization. All living matter has a characteristic organization occurring in several levels. Basic to all living organisms is the cell, composed of protoplasm, cytoplasm, and nucleus or nuclear material. Cells combine into structural units called organs in the larger organisms.

The total of all the chemical processes going on in "living" matter is metabolism. This attribute allows the organism to consume food material, utilizing that which is needed and discharging the waste product. When the constructive or synthetic processes exceed destructive metabolism growth occurs. After a cell has grown to a characteristic size, it divides forming two cells, the process is called reproduction. When only one individual is involved it is referred to as asexual reproduction. Sexual reproduction takes place when two individuals are involved.

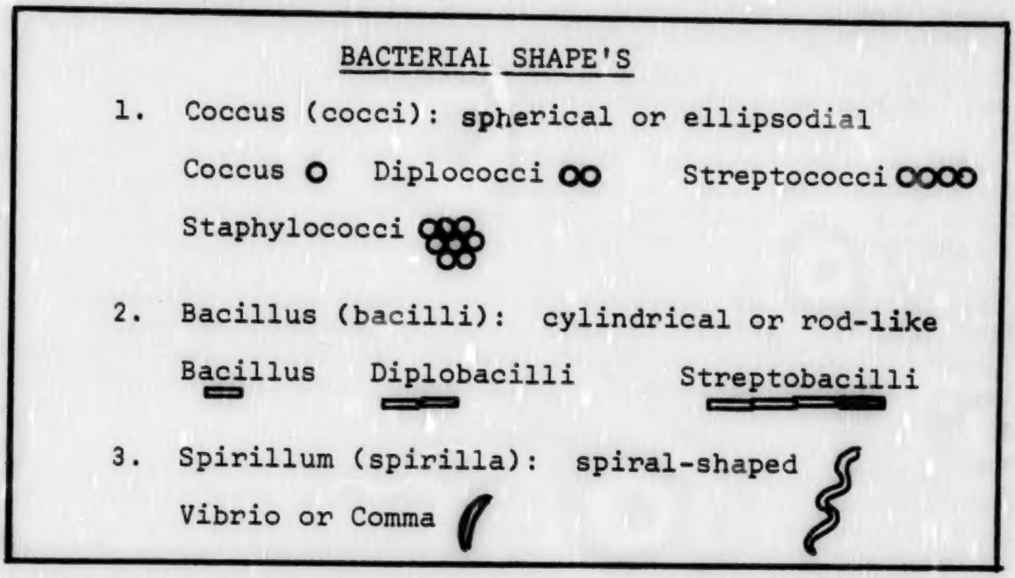
The ability of a "living" organism to respond to external stimuli such as pressure, temperature, light and so forth, is referred to as irritability. The fitness of an organism for the environment in which it lives is called adaption. This attribute develops through various evolutionary mechanisms, and becomes permanent through inheritance.

BACTERIOLOGY

Bacteriology deals with the study of microorganisms known as bacteria. It includes a large group of typically unicellular microscopic organisms widely distributed in air, water, soil, the bodies of living plants and animals, and dead organic matter. The so-called true bacteria are in a class by themselves, neither plants nor animals. Some higher bacteria border on the plants and are more related to them; other higher bacteria border on the animals. For this reason it is not possible to classify bacteria with the plants or animals. They are sometimes placed in a third kingdom, intermediate between plants and animals. For uniformity , bacteria are still placed in the plant kingdom. It is quite possible that in future classifications the bacteria will be separate from the plants and made a kingdom equal in rank to the plant and animal kingdoms. Bacteria do not exist as parts of organisms, only as single cells.

Bacteria are found everywhere in nature - in soil, air, and water. Bacteria are found in the human body. They manufacture vitamins and help break down food and are eliminated with waste. products and decompose organic matter. Bacteria known as saprophytes live on dead organic matter, while others subsist on living matter and are called parasites, which can be pathogens. Pathogenic bacteria cause disease and are the reason for chlorinating drinking waters and wastewater effluents. Saprophytic organisms are the most prevalent bacteria and are responsible for the organic decomposition in wastewater treatment.

There are thousands of types of bacteria, and they are classified according to various characteristics, one of which is shape. There are three basic shapes, a spheroidal cell is called a coccus; a cylindrical cell (the most numerous in wastewater plants) is called bacillus; and the spiral shaped bacteria known as spirillum. Bacteria of each shape can appear in different groupings.



BACTERIAL SHAPE'S

1. Coccus (cocci): spherical or ellipsodial

Coccus Diplococci Streptococci

Staphylococci

2. Bacillus (bacilli): cylindrical or rod-like

Bacillus Diplobacilli Streptobacilli

3. Spirillum (spirilla): spiral-shaped

Vibrio or Comma

Bacteria can also be described by their growth requirements. Like other living organisms, bacteria have certain requirements for growth. The most important factors influencing are food and sufficient moisture to carry food into the cell. Bacterial growth is also sensitive to temperature and each type has an air optimum temperature at which its growth is greatest. A few organisms known as psychrophiles are capable or growing at 5°C (41°F) or less; those that grow best between 25°C (77°F) and 40°C (104°F) are called mesophiles; and those with an optimum temperature range of 50°C (122°F) to 55°C (131°F) are called thermophiles. Most organisms perfer a pH range of 6.5 to 8.0 for optimum growth. There are exceptions. An organism involved in sewer pipe corrosion can grow at a pH of 1.0. Besides pH and temperature factors, bacterial growth is also influenced by oxygen availability. Those organisms that require free oxygen for growth are called aerobes; those that grow without free oxygen are known as anaerobes. Facultative organisms are a type that can adapt to either environment.

Many other conditions affect the growth and survival of bacteria. Included are light, heat, drying, bacterial agents, bacteriostatic agents, and antimetabolites of various kinds.

The heat requirement for optimum growth of bacteria varies with the species and was discussed previously. Bacteria are not destroyed by low temperatures. Most bacteria reproduce very slowly or not at all under these conditions. When transferred from the frozen state to a more suitable environment, they can carry on a normal life cycle. Extreme heat destroys all bacterial species, although those which are in the spore stage will be capable of withstanding much more heat than those which are not. Moist heat or steam is more efficient in destroying bacteria than dry heat.

Certain wave-lengths of light are destructive to bacterial cells. Those that lie in the ultra-violet region of the spectrum are especially destructive. For destruction to occur, the light ray must strike the cell directly.

Bacteria cannot reproduce without moisture. Drying, or dessication, of food materials is therefore a method for its preservation from decomposition by bacteria. Spore-forming bacteria may survive in a dry environment but they cannot function normally.

A germicide is a substance which destroys a bacterial cell on contact, and a bacteriostatic agent is a substance which prevents the cell from reproducing. A bacteriostatic agent indirectly brings about the destruction of the bacterial culture, since without reproduction there can be no continuance of life. The best known germicide in the water and wastewater field is chlorine.

Antimetabolites are substances which destroy or alter metabolic agents or growth factors essential to the normal life processes of a bacterial cell. Without normal growth and reproduction life ceases. Some of the antibiotic drugs used in treating disease are antimetabolites.

Bacteria are identified through a systematic application of procedures which are designed to (1) secure a "culture" of bacteria, that is a very large number of living bacterial cells in or on a medium which provides adequate food for them, (2) by successive sub-culturing, secure a separation of the individual species from each other, (3) determine the cultural characteristics of each species, (4) determine the morphological characteristics of each species by examination of stained preparations of bacterial cells.

There are many kinds of bacterial cultures, e.g. growth in solutions or liquid suspensions of the nutrient materials, growth in suspensions of the nutrients in jelly-like substance such as agar or gelatin, growth on the surface of animal or vegetable tissue, and growth on the flesh or in the blood stream of animals. There are a host of materials used as nutrients, ranging from simple inorganic salts to organic carbohydrates such as sugars, to relatively simple protein substances such as egg albumin, and to the extremely complex proteins of animal tissue.

Sub-culturing is the process of securing growth of the bacteria in colonies and "fishing" a colony for regrowth on fresh medium. In fishing, a sterile wire is gently touched to a surface of the colony thus removing a few cells which cling to the tip of the wire. These are then immediately transferred to a fresh lot of sterile medium in another vessel. Incubation of the transplants then produces new colonies. A successive number of such transfers eventually produces a "pure" culture, that is a culture of a single species.

Small amounts of the pure cultures are inoculated into specialized media to determine growth characteristics. These media often consist of carbohydrates of various kinds differing in chemical structure and complexity. Growth is usually indicated by production of a cloudy effect. The number of suspended cells increases and acid and/or gas are produced as the carbohydrates are decomposed by the bacteria.

A wide variety of stains and staining techniques are available to enable the bacteriologist to observe size, shape, spore formation, presence of flagella, etc. under the microscope.

As a basis for identification, the bacteriologist draws upon the written record of the observations made by generations of his predecessors on thousands of bacterial species.

Most waters contain large numbers of bacteria. The numbers may vary depending upon the source with water polluted by wastewater containing as many as thousands or even millions per milliliter. There are at least five diseases of bacterial origin which can be transmitted through wastewater contaminated water. It is impractical to ascertain the safety of a water by analysis for each type of pathogenic bacteria. Most pathogens are very difficult to isolate and an excessive amount of time would be required to complete such an analyses. A more practical scheme is to examine the water for the presence or absence of an organism or group of organisms specifically identified with wastewater. The coliform group of bacteria, whose normal habitat is the large intestine of man, conforms to this requirement. These bacteria, of which there are more than 30 individual species, conform to the requirements of an ideal indicator of wastewater contamination which are:

1. Always present when wastewater is present
2. Always absent when wastewater is absent
3. Survives longer than most pathogenic species
4. Easily isolated and identified

The coliform group of bacteria are specifically "all the aerobic and facultative anaerobic, Gram-negative, non-spore forming, rod shaped bacteria which ferment lactose with gas formation with 48 hours at 35°C.[*] There are two bacteriological methods available for estimating the degree of wastewater contamination - the multiple tube fermentation method and the membrane filter method.

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[*] APHA, AWWA, WPCF. Standard Methods for the Analysis of Water and Wastewater, 1975, 14th Ed., New York.

OPERATING PROCEDURES	STEP SEQUENCE	INFORMATION/OPERATING GOALS/SPECIFICATIONS	TRAINING GUIDE NOTES
1. Preparation of EC Broth	1. Weigh 1.85 grams of dehydrated EC Broth. Close cover of bottle of dehydrated medium tightly after removal.	1a. Dehydrated media take moisture out of air, become caked. 1b. Caked media unsatisfactory; discard. 1c. This amount is sufficient for 5 tubes.	
	2. Dissolve in 50 ml distilled water.	2a. Gentle heat if necessary. No boiling.	
	3. Place 10 ml of the solution of prepared EC Broth in each culture tube.	3a. Use 150 x 22 mm tubes. 3b. 10 ml pipet, automatic pipetter, or funnel and pinchcock are acceptable. 3c. Accuracy of delivery: ± 0.5 ml.	
	4. Insert one fermentation vial into each tube of medium, open end down.	4a. Tubes and vials previously washed as indicated. 4b. Use 75 x 10 mm tubes.	
	5. Place tube cap on each tube of culture medium.	5a. After all tubes have been filled.	
	6. Sterilize in autoclave.	6a. After all tubes have been filled. 6b. Sterilization at 121°C for 15 minutes. 6c. Medium must be removed from autoclave as soon as possible after pressure has returned to normal.	

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GLOSSARY

Aerobe - An organism that grows best in the presence of free oxygen.

Anabolism - The constructive process by which simple substances are converted by living cells into more complex compounds.

Anaerobe - An organism which can grow without either air or free oxygen.

Antibiotic - Literally, growth-inhibiting; usually denotes bacteriostatic substance produced by microorganism.

Antiseptic - A substance that prevents or inhibits the growth of microorganisms without necessarily destroying them.

Asepsis - Absence of septic matter or freedom from microorganisms.

Autoclave - An apparatus for sterilizing by steam under pressure.

Autotrophic - Capable of living on inorganic matter.

Baccilus - A rod-shaped bacterium; a genus of rod-shaped bacteria of the family Bacteriaceae.

Bacteria - Minute, one-celled, microscopic, plantlike organisms which multiply by fission and lack chlorophyll.

Bactericide - Any agent that destroys bacteria.

Binary fission - A form of asexual reproduction involving simple cell division of the cytoplasm and nucleus, if present, into two equal parts.

Catabolism - The process of destruction or breakdown of tissue and cells from complex to simpler compounds.

Catalyst - A substance that accelerates a chemical or physical reaction without itself being destroyed or changed.

Coccus - An organism which is round or spherical in shape.

Colony - A group of bacteria, on a solid medium, usually derived from the multiplication of a single organism and visible to the naked eye.

Commensalism - Living together of two species, one of which is benefited by the association while the other is apparently neither benefited nor harmed.

Culture - A growth of microorganisms.

Culture medium - Any substance or preparation suitable for and used for the growth and cultivation of microorganisms.

Decay - Decomposition of organic compounds under aerobic conditions to form amino acids which are then further broken down.

Desiccator - A closed vessel for apparatus or chemicals that are to be dried and kept free from moisture; usually contains a dehydrating agent.

Dye - A material used for staining or coloring, consisting of benzene rings with chromophore and auxochrome groups.

Enteric - Pertaining to the intestines.

Enzyme - An organic catalyst produced by a living cell.

Facultative aerobe - A microorganism which is fundamentally an anaerobe but can grow in the presence of free oxygen.

Facultative anaerobe - A microorganism which is fundamentally an aerobe but can grow in the absence of free oxygen.

Fermentation - Incomplete oxidation of carbohydrates and carbohydrate-like compounds by microorganisms.

Flagella - Whiplike processes for motility.

Formaldehyde - HCHO; a disinfectant gas with a pungent odor.

Formalin - A 40% solution of gaseous formaldehyde.

Fungi - A subphylum of plants which do not contain chlorophyll, including bacteria, yeast, and molds.

Germs - A microbe or bacillus.

Germicide - Synonymous with disinfectant.

Gram-negative bacteria - Bacteria which lose the initial stain of the Gram stain, are decolorized, and take the color of the final stain.

Gram-positive bacteria - Bacteria which take the initial stain of the Gram stain and are not decolorized, so that they appear purple.

Incubator - An apparatus for maintaining a constant and suitable temperature for the growth and development of a bacterial culture or other materials.

Indicator - A substance, usually a weak organic acid or base, which changes color when the reaction of a solution changes.

Mesophilic bacteria - Bacteria that grow best at moderate temperatures.

Metabolism - The sum of all the physical and chemical processes by which the tissues are formed and maintained and energy is made available for use by the body.

Micromicron - The millionth part of a micron or 10^{-10} cm., designated by the symbol $\mu\mu$.

Micron or micromillimeter - One-millionth part of a meter, or one-thousandth part of a millimeter, or 1/25,000 of an inch, designated by the Greek letter μ.

Mixed culture - Growth of two or more organisms in the same medium.

Morphology - The science of the form and structure of organized beings.

Mycology - The science and study of fungi.

Obligate aerobe - An organism which must have free oxygen for its growth.

Obligate anaerobe - An organism which can live and grow only in an environment with no or minimal amounts of free oxygen.

Osmosis - Passage through a membrane; when two solutions of unequal density are separated by a membrane which selectively prevents the passage of solute particles but is permeable to the solvent, pure solvent passes from the lesser to the greater concentration.

Parasite - A plant or animal which lives upon or within another living organism at whose expense it grows without giving anything in return.

Pathogens - Disease-producing microorganisms.

Phenol - Carbolic acid, a colorless crystalline compound C_6H_5OH, obtained by the distillation of coal tar and having strong antiseptic and disinfectant properties.

Phenol coefficient - A number indicating the relative efficiency of disinfectants. It is the quotient obtained by dividing the highest dilution of a disinfectant which kills a test organism in a fixed time by the highest dilution of phenol showing the same results.

Protozoa - Unicellular, nucleated, animal organisms with diversification and specialized functions of the protoplasm.

Psychrophilic bacteria - Cold-loving bacteria whose optimum temperature for growth is 15° - 20°C or below.

Pure Culture - Specific bacterial growth of only one type of organism.

Putrefaction - The decomposition of animal or vegetable matter in the absence of oxygen and characterized by the formation of amino acids, mercaptans, indol, skatol, and hydrogen sulfide, with an accompanying unpleasant odor.

Stab Cultures - Cultures in which the organisms are inoculated far down into the solid butt of the medium to allow for possible anaerobic growth.

Stain - Any dye, reagent, or other material used in coloring tissues or organisms for microscopic study.

Sterilization - The process of freeing completely from all living microorganisms.

Streak - Inoculation of slant or plates with a streak or a direct line movement across the surface of culture media.

Symbiosis - The living together or close association of two dissimilar organisms with mutual benefit.

Thermal death point - The temperature which destroys all the bacteria present within a given time.

Thermal death time - The length of time required to kill all the organisms in a given substance at a given temperature.

Thermophilic bacteria - Bacteria which grow best at a temperature of 50° - 55°C.

Transfer of cultures - Transplanting viable bacteria from an old medium to a fresh new one.

MODULE IV

LABORATORY INVENTORY

ORDERING EQUIPMENT AND SUPPLIES

One of the areas that is often overlooked in chemical laboratories is the equipment and supplies that are needed. For the parameters that are required in the permit, there is a listing of all equipment and supplies that are required. These are broken down into three catagories:

a. Capital Equipment
b. Reusable Supplies
c. Consumable Supplies

Capital Equipment is usually equipment purchased on a one-time basis and is $100.00 or more.

It should be noted that some communities or agencies may have other set figures for distinguishing capital equipment from reusable supplies.

Reusable supplies are usually less than $100.00 but will have to be replaced periodically because of breakage.

Consumable supplies are the supplies that will be depleted by everyday use.

An example of a supply list for the Fecal Coliform Test - Multiple Tube Dilution Method is given on the following pages.

BASIC LABORATORY SKILLS: Fecal Coliform Test by the Multiple Dilution Tube Method

General Description of Equipment Used in the Test Analysis

A. Capital Equipment:

1. Autoclave, providing uniform temperatures up to and including 121°C, equipped with an accurate thermometer, pressure gauges, saturated steam power lines and capable of reaching required temperature within 30 minutes.
2. Balance, 0.1 g. sensitivity at load of 160 g.
3. Air incubator to operate at 35°C ± 0.5°C.
4. Incubator, waterbath, to operate at 44.5°C ± 0.2°C and to accommodate tube racks as described separately.
5. Oven, *hot-air sterilizing, to give uniform temperatures and with suitable thermometer to register accurately in range of 160-180°C.
6. pH meter, accurate to at least 0.1 pH unit, with standard pH reference solution(s).
7. Water distillation apparatus (glass or block tin), or source of distilled water suitable for bacteriological culture media.

B. Reusable Supplies:

1. Apron or coat suitable for laboratory
2. Baskets, wire for discarded cultures
3. Bottles, dilution*, 6-oz. screw caps, with 99 ml volume level etched on one side.
4. Bottles, sample*, 250 ml (6-8 oz.), wide mouth, glass stopper preferred.
5. Bottle, squeeze type, with disinfecting solution
6. Burner, gas, Bunsen burner type
7. Cans, pipet, aluminum or steel; not copper. (If plastic, or other type of disposal pipets are used, this item is unnecessary.)
8. Metal caps* to fit 20 x 150 mm culture tubes
9. Jar, to receive discarded pipets
10. Inoculation loop, 3 mm diameter loop of nichrome or platinum-iridium wire, 26 B&S gauge, in holder.
11. Pipets*, 1 ml, with 0.1 ml graduations, Mohr type preferred, sterile, cotton plugged, glass or disposal plastic
12. Racks, culture type* 10 x 5 openings, to accept tubes at least 20 mm in diameter
13. Sponge, for cleaning desk top
14. Tubes, culture*, 20 x 150 mm.
15. Tubes, fermentation*, 12 x 75 mm vials to be inverted in culture tubes

C. Consumable Supplies: (must be replaced when stocks get low)

1. Distilled water, suitable for bacteriological cultures (note distillation apparatus required in capital equipment.)
2. EC Broth, dehydrated (recommend purchase of 1-lb units)
3. Lactose Lauryl Sulfate Tryptose Broth, dehydrated (recommend purchase of 1-lb. units)
4. Potassium Dihydrogen Phosphate (KH_2PO_4) (recommend purchase of 1-lb. units)
5. Disinfectant, for bench tops. (Use household bleach solution prepared according to instructions on bottle.)
6. Wax pencils (recommend soft wax equivalent to Blaisdell 169T)

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* Items marked are needed in quantities or require size or space allowances which cannot be specified here, as they vary according to the daily analysis schedule. As a rule-of-thumb, space/size or quantity requirements should be at least 3 times the normal daily requirements. For further information on specifications for equipment and supplies, see the Microbiology Section of the current edition of "Standard Methods for the Examination of Water and Wastewater."

NAME ______________________

ASSIGNMENT #1: Equipment and Supplies for Fecal Coliform Test - Multiple Dilution Tube Method

Assume that you are responsible for the effluent monitoring tests for your treatment plant, which has an average daily flow 18 mgd. In addition, you conduct the effluent monitoring tests for 2 small plants in your vicinity, each of which has an average daily flow between 1 and 4.99 mgd.

Prepare a purchase request, based on information in catalogues made available to you by your instructor, for the items shown below. (The amount should be enough to last one year. The size of fixed equipment should be appropriate to the amount of lab work required; assume that the sampling schedules will be set up to have the work from the surrounding laboratories come in on a scheduled basis to provide an even daily workload.)

Order: Wax pencils

EC Broth (dehydrated)

Fermentation Tube Assemblies

Autoclave

Minimum information in the purchase request should identify --

a. Yourself, as the originator of the request

b. The name of the item(s) requested

c. The size, and number of the item(s)

d. The cost per unit and the total cost for the item

e. The name of the catalogue from which you obtained the item, the stock number of each item selected from the catalogue, and any other identifying information that the purchasing officer should have.

NAME ____________________

ASSIGNMENT #2: Equipment and Supplies for Fecal Coliform Test - Multiple Dilution Tube Method

Assume that you are responsible for the effluent monitoring tests for your treatment plant, which has an average daily flow of 45 mgd. In addition, you conduct the effluent monitoring tests for 3 small plants in your vicinity, each of which has an average daily flow between 1 and 4.99 mgd.

Prepare a purchase request, based on information in catalogues made available to you by your instructor, for the items shown below. (The amount should be enough to last one year. The size of fixed equipment should be appropriate to the amount of lab work required; assume that the sampling schedules will be set up to have the work from the surrounding laboratories come in on a scheduled basis to provide an even daily workload.)

Order: Pipets

Lactose Lauryl Sulfate Tryptose Broth (dehydrated)

Water Bath Incubator

Laboratory Aprons or Coats

Minimum information in the purchase request should identify --

a. Yourself, as the originator of the request

b. The name of the item(s) requested

c. The size, and number of the item(s)

d. The cost per unit and the total cost for the item

e. The name of the catalogue from which you obtained the item, the stock number of each item selected from the catalogue, and any other identifying information that the purchasing officer should have.

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